Return to NINDS Parkinson's Disease Research Web

Life and Death of Neurons

Principal Investigator: ABELIOVICH, ASA
Grant Number: 5R01NS046659-02
Title: Molecular and Cellular Analyses of Parkin Function

Abstract: Defective protein degradation through the ubiquitin proteasome pathway (UPP) has been hypothesized to play a central role in neurodegenerative disorders such as Parkinson's disease (PD). Mutations in Parkin, a putative ubiquitin ligase component, cause a familial, autosomal recessive form of PD characterized by midbrain dopamine neuron loss. It has therefore been hypothesized that inefficient degradation and consequent toxic accumulation of Parkin ubiquitination substrates underlie the loss of dopamine neurons in autosomal recessive Parkinson's disease. We further hypothesize that Parkin may play a direct role in regulating neuronal survival in the CNS. We propose to use molecular and cellular tools to investigate the mechanism of Parkin action in protein ubiquitination and neuronal survival. Our preliminary data indicate that Parkin associates in a multiprotein ubiquitin ligase complex with 2 previously characterized ubiquitin ligase components, the F-box/WD repeat-containing protein hSel- 10, and Cullin-1 (Cul 1). Furthermore, hSel-10 serves to direct this complex to specific substrates including Cyclin E, a putative regulator of neuronal apoptosis. We will test the hypotheses that (1) auxiliary components of the Parkin ubiquitin ligase complex serve to regulate or target this activity, and (2) that, in Parkin-associated familial PD, premature neuronal death is a consequence of defective ubiquitination and the accumulation of neuronal apoptosis-related Parkin complex substrates. -

Principal Investigator: ALBUQUERQUE, EDSON
Grant Number: 5R01NS041671-04
Title: Nicotinic Receptors in Septally Innervated Hippocampus

Abstract: The dysfunction and degeneration of the nicotinic cholinergic system in the brain are integral physiopathological indicators of one of the most socially impacting neurological disorders, Alzheimer's disease (AD). In AD, the permanent loss of cholinergic neurons and nicotinic receptors (nAChRs) in brain areas that process cognitive functions, particularly the hippocampus and the frontal cortex, correlates well with the decline in cognition and memory. To date, treatment of patients with AD relies heavily on the use of acetylcholinesterases. These drugs, by increasing function of the cholinergic system, partially reverse the symptoms of AD patients. Recently, clinical trials have shown that nicotinic agonists (including nicotine) and drugs that allosterically potentiate the activity of nAChRs are more effective for treatment of patients with AD. The mechanisms underlying the effectiveness of these drugs remain unknown, because there is very little information on how function and expression of neuronal nAChRs in the brain are regulated by cholinergic afferents. In addition, detailed analysis of regulation of nAChR expression and function in the hippocampus by septal cholinergic afferents has been limited by the lack of a viable biological preparation that closely resembles the nicotinic cholinoceptive hippocampal system in vivo. Our initial characterization of the nicotinic properties of hippocampal neurons in organotypic, hippocampal and septal-hippocampal cultures constitutes the mainstay of the present proposal, as it establishes the septal-hippocampal co-cultures as an excellent model for in vitro study of the influences of septal innervation on nAChR expression in the hippocampus. Thus, this proposal is designed to use convergent, multidisciplinary approaches to address the central hypothesis that septal innervation and nicotine dynamically modify the hippocampal cholinergic system. The first goal of this study is to use electrophysiology, confocal microscopy, ligand binding and immunocytochemistry to determine whether septal innervation alters the nicotinic properties of different types of hippocampal neurons during development in organotypic cultures. The second goal is to use electrophysiological assays, recombinant DNA technology and "knock-out" mice, which have a null mutation in the gene encoding alpha7 nicotinic receptors, to study nAChR targeting and to investigate the motifs in the nAChR subunits that account for final receptor targeting in hippocampal neurons. The final goal is to use electrophysiological, biochemical and molecular biological techniques to evaluate how nicotine affects alpha7 and alpha4beta2 nAChR expression in the hippocampus. The results of these studies will have far

Principal Investigator: ALEXANDRE, LUCIEN
Grant Number: 5F31NS044583-03
Title: Minority Predoctoral Fellowship

Abstract: As I am in the very early stages of the MST program, I have yet to select a thesis laboratory or complete my laboratory rotations. However, I have identified several laboratories whose research interests parallel my own, i.e., studying the mechanisms behind and understanding the onset of neurodegeneration. One such laboratory with whom I completed a rotation this summer is embarking on a very exciting new direction in the study of neurodegenerative diseases as a function of early determination in neural stem cells rather than being an age related phenomenon. Dr. Mehler's studies are aimed at identifying which stage of development is instrumental for the commitment to the brain cell population that will undergo cell death later in life. These studies and the means for answering such revolutionary questions will encompass the latest in dissociated cell culture, molecular biology, and genetic analysis in order to identify the origins of diseases such as Alzheimers, Parkinson and Huntingtons diseases. Should these experiments prove fruitful this would provide a novel approach to our understanding of neurodegenerative diseases and open the gates to new treatments and screening for individuals early in life even before the onset of disease.-

Principal Investigator: ARANCIO, OTTAVIO
Grant Number: 5R01NS040045-05
Title: Role of Presynaptic Proteins in Transmitter Release

Abstract: The overall purpose of this project is to gain a better understanding of the mechanisms underlying neurotransmitter release. Its modulation is thought to play an important role in higher order mental process such as learning and memory. Studies in vivo or in slices have allowed limited advances in the analysis of synaptic release properties, because of the lack of direct access to the presynaptic terminal. Cultured systems have real advantages over intact animals and slices, including the possibility of delivering drugs intracellularly to either side of the synapse including the presynaptic terminal, and the possibility of long-term access to cells under controlled environment for genetic manipulation. Thus, cultured hippocampal neurons will be used in our experiments. Our researches will focus on cGMP-dependent protein kinases (cGKs), a family of proteins with a poorly understood function, and their action on transmitter release. The first specific aim will examine whether presynaptic cGK type I and II are involved in basal transmitter release and plasticity. The goal of the second specific aim will be to test whether VASP and CAMKII, two cGK substrates, are cGK targets during neurotransmission and plasticity. cGK involvement in the release machinery and vesicle cycle kinetics will be examined in the third specific aim in order to explore the functional role of the kinase. Finally, cGK regulation of the expression of the presynaptic proteins, synaptophysin and a-synuclein, will be examined by combining immunocytochemical techniques with electrophysiological recordings. Evidence for a mechanistic link between cGKs, increase in the number of synaptophysin and a-synuclein immunoreactive clusters and long-lasting increase in neurotransmitter release will be provided. A better understanding of these mechanisms will yield new drug therapies with potential in treatment of Alzheimer's Disease and other neurodegenerative disorders with staggering social, economic and personal costs to the sufferers, their families and all of society. -

Principal Investigator: Bakay, Roy A
Grant Number: 1R01NS046612-01A1
Title: Stem Cells in CNS Transplantation

Abstract: Stem cells offer tremendous promise for the future of transplantation. We propose examining embryonic stem cells (ESC) in monkey allografts. We will compare dopaminergic enriched ESC to fetal mesencephalic (FM) neurons in their ability to survive, innervate, and restore lost function in the best animal model of PD, the MPTP treated monkey. The primate is essential for this study to test the hypothesis that replacement strategy must completely reinnervate the very large volume of the monkey striatum. Recently clinical trials have indicated that dopaminergic (DAergic) replacement with FM neurons can cause severe debilitating dyskinesia. It is then imperative to have a clear understanding of how a DAergic enriched ESC replacement strategy affects l-dopa-induced dyskinesia (LID). In this regard, we will also compare the effects of FM transplants and DAergic enriched ESC upon the dyskinesia profile of MPTP monkeys. The potential to induce or diminish dyskinesia will be tested with the best model of dyskinesia (primate LID model). The key problem of parkinsonian transplantation with fetal or stem cells grafts is the incomplete reinnervation of host striatum. Like the FM transplant patients, focal areas of relative hyperdopaminergic activity should render these monkeys highly susceptible to LIDs. Thus to optimize reinnervation and functional recovery while minimizing the potential for dyskinesia, we will also treat DAergic enriched ESC with glial cell line-derived neurotrophic factor (GDNF) delivered via a lentiviral vector. The lenti-viral vector is critical to this hypothesis because of the proven ability to transfect the entire striatum and act not as a point source but as a volume source to stimulate reinnervation. Intraparenchymal GDNF released diffusely throughout the entire striatum should act as a developmental cue for these immature cells to extend DAergic processes throughout the striatum as well as provide neuronal rescue for dopaminergic neurons in the pars compacta of the substantia nigra. Sufficient subjects and multiple controls are included to insure proper interpretation of the data. The present series of experiments serves to provide the essential preclinical data needed to help determine the utility of nonhuman dopaminergic enriched stem cells. -

Principal Investigator: BAUDRY, MICHEL
Grant Number: 1R01NS048521-01A1
Title: Calpain inhibitors in models of Parkinson's disease

Abstract: Parkinson' s disease is a neurodegenerative disease that specifically affects dopaminergic neurons in the substantia nigra. Although several hypotheses have been proposed to account for the specificity of the neurodegenerative features of the disease, the exact cause of the disease remains to be elucidated. Significant advances in our understanding of the possible causes of the disease were provided by the serendipitous discovery that a neurotoxin, 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP), elicits a pattern of neurodegenerative features in humans and experimental animals identical to that seen in patients with Parkinson' s disease. A potential target to prevent neurodegeneration in Parkinson' s disease is the calcium-dependent protease calpain. Calpain levels are elevated in post-mortem substantia nigra of patients with Parkinson' s disease, MPP+ neurotoxicity in granule cell cultures is associated with calpain activation and blocked by calpain inhibitors, and calpain has been implicated in several neurodegenerative diseases. We have recently obtained a series of novel and potent calpain inhibitors and have demonstrated their potency in preventing NMDA-induced calpain activation in cultured hippocampal slices. The current proposal is aimed at testing the hypothesis that calpain activation plays a critical role in animal models of PD and that calpain inhibitors are neuroprotective in these models. We will first determine the potency and efficacy of calpain inhibitors to prevent MPTP toxicity in cultured slices from rat mesencephalon. We will then use structure activity relationship in conjunction with additional assays to identify the best inhibitors to be tested in in vivo models. Finally, we will test the hypothesis that calpain is activated and that calpain inhibitors are neuroprotective against MPTP-mediated neurotoxicity and behavioral impairments in vivo in C57Bl/6 mice, and against rotenone-mediated neurotoxicity in rats. Conversion of the pro-apoptotic factor Bid to its active, truncated form tBid will be tested as part of the mechanisms by which calpain activation induces cell death. These studies will test the hypothesis that calpain inhibitors might prevent neurodegeneration not only in Parkinson' s disease but also in a variety of conditions resulting from exposure to environmental toxins. Finally, because calpain has also been implicated in the mechanisms underlying Amyotrophic Lateral Sclerosis (ALS), our proposal could lead to significant advances in the treatment of this neurodegenerative disease as well. -

Principal Investigator: BENNETT, JAMES P
Grant Number: 5R01NS039005-05
Title: OXIDATIVE STRESS IN PARKINSON'S DISEASE

Abstract: Idiopathic Parkinson's Disease (PD) is a major neurodegenerative disease affecting at least 1 million Americans, and the cellular cause of PD is not yet known with certainty. This proposal will explore further the central hypothesis that defects in mitochondrial electron transport chain (ETC) function are a major contributor to premature cell death in PD and will address four Specific Aims 1) define the pathophysiology of mitochondrial transition pore function, and how regulation of membrane potential and intracellular calcium signaling are altered in PD; 2) determine mechanisms of Bcl protein regulation in PD cybrids, and whether transfection with Bcl-overexpression vectors alters mitochondrial function and improves survival; 3) further define the interactions among MAPKinase signaling pathways and NFkappaBeta transcription factor in PD; and 4) characterize mitochondrial transition pore complexes isolated from human postmortem PD brain and compare their function to those isolated from control brain. This project will make use of state-of-the- art intracellular ion imaging technology, RT-PCR techniques, gene transfection strategies, and will develop cell-free systems to examine several inter-related hypotheses. Behind all of these laboratory experiments is a therapeutic imperative, which will be explored in cell and cell-free models. Because new data presented in this application supports the hypothesis of systemically increased oxidative stress in PD patients, exploring these events in an established cell model is even more compelling. This proposal will also compare findings in PD cybrids with those in SY5Y cells exposed to chronic rotenone treatment, a pharmacological cell-based model of complex I loss. Ultimately, the results from this proposal will establish the central importance of genetically acquired mitochondrial ETC dysfunction as an etiologic factor in sporadic PD. Paradigms for evaluating neuroprotective therapies will also be developed to allow targeted approaches to correcting consequences of increased oxidative stress in cells. -

Principal Investigator: Berry, Marla J
Grant Number: 2R01NS040302-06
Title: Selenoprotein P function and regulation of expression

Abstract: There is a wealth of information implicating cumulative cellular injury inflicted by reactive oxygen species and heavy metal toxicity in neuronal damage and neurodegenerative diseases. Cells have evolved various endogenous antioxidant defenses to afford protection from oxidative injury or to reduce oxidative stress. Selenium is an essential contributor to these defenses, as it is required for the activity of a family of antioxidant enzymes that protect cells against the damaging products of normal oxygen metabolism. Selenium has also long been known to function as an antidote to toxicity of heavy metals. Selenoprotein P has recently been shown to function as a selenium delivery protein to brain, providing a source of this essential trace element for synthesis of other selenoproteins when selenium is deficient in the diet. Targeted disruption of the selenoprotein P gene results in neurological dysfunction. The overall goals of this study are to investigate the selenium delivery function of selenoprotein P in cells of neuronal origin, and to identify the crucial target selenoproteins which function in protection from oxidant and heavy metal induced damage, and which presumably explain the neurological effects of selenoprotein P gene disruption. These goals will be addressed through the following specific aims: 1. Investigate the means by which selenoprotein P serves as a Se donor to cells in culture, including interactions at the cell membrane and within the cell. 2. Investigate the expression levels and subcellular localization of specific selenoproteins, and whether localization or expression levels or patterns change in response to oxidative damage. Investigate the expression of specific selenoproteins in tissue sections from different brain regions in mice, and the changes in the expression levels or localization in response to GSH depletion or ischemia/reperfusion injury. 3. Identify the specific selenoproteins in neuronal cells responsible for protection from oxidative damage, resulting from either reactive oxygen species production or accumulation, or heavy metal induced damage. 4. Investigate expression of selenoproteins in brain sections obtained at autopsy from a cohort of Japanese men diagnosed with Alzheimer's or Parkinson's disease, vascular dementia, other neurological damage or with no evidence of neurodegenerative disease. -

Principal Investigator: BING, GUOYING
Grant Number: 5R01NS044157-02
Title: Cox-2 deficient mice are resistant to MPTP neurotoxicity

Abstract: Parkinson's disease (PD) is a movement disorder characterized by the progressive loss of dopamine-containing neurons in the substantia nigra pars compacta (SNpc). Loss of SNpc dopaminergic neurons results in the depletion of striatal dopamine levels and produces symptoms such as tremor, muscle rigidity, and bradykinesia. The etiology of PD is unknown, but chronic inflammatory processes, microglial activation, and oxidative stress are thought to play prominent roles in the degeneration of dopaminergic neurons in the SNpc. Microglia are thought to contribute to neurodegeneration by releasing cytotoxic agents such as pro-inflammatory cytokines and reactive oxygen species that increase inflammation and oxidative stress. N-Methyl-4-phenyl-1,2,3,6,-tetrahydropyridine (MPTP) is a neurotoxin found to mimic many of the features of PD in animal models, including loss of dopaminergic neurons in SNpc and activation of microglia. Recent observations indicate that cyclooxygenase-2 (COX-2) deficiency in mice reduces the susceptibility of SNpc dopaminergic neurons to MPTP toxicity and diminishes MPTP-induced microglial activation. The purpose of this study is to test the hypothesis that COX-2-regulated inflammatory processes exacerbate MPTP neurotoxicity by activating microglia and increasing oxidative stress that contributes to the degeneration of dopaminergic neurons in the SNpc. To test this hypothesis, mice deficient in the COX-2 gene will be treated with MPTP to determine the role of COX-2 in MPTP-induced neurodegeneration. Furthermore, wild-type mice will be administered exogenous COX-2 inhibitors prior to MPTP treatment to evaluate the protective effects of COX-2 inhibitors against MPTP neurotoxicity. Following these experiments, dopaminergic neuron survival, microglial activation, striatal dopamine levels, and functional recovery will be assessed. In addition, protein modification, generation of reactive oxygen species, expression of inflammatory cytokines and apoptosis-related genes, and activation of specific signaling molecules will be evaluated to determine the molecular mechanisms by which COX-2 exacerbates MPTP neurotoxicity. The goals of this study are to elucidate the changes in inflammatory processing affected by COX-2 deficiency, to explore the etiology and molecular mechanisms underlying Parkinsonian symptoms in the experimental MPTP model, and to develop novel therapeutic treatments for PD and other neurodegenerative diseases.-

Principal Investigator: Blackstone, Craig
Grant Number: 5Z01NS002992-03
Title: Molecular Pathogenesis Of Hereditary Neurological Disorders

Abstract: Unavailable

Principal Investigator: BODELL, WILLIAM J
Grant Number: 5R01NS041939-03
Title: DNA Adducts Formed by Dopamine

Abstract: (Adapted from applicant's abstract): The pathophysiological processes responsible for the development of Parkinson's disease has been identified as a loss of dopaminergic neurons in the pars compacta of the substantia nigra. Despite intensive study, the molecular mechanisms responsible for the selective loss of these neurons remains unknown. An intriguing feature of these neurons is the presence of neuromelanin. The biological function of neuromelanin in these cells is unknown and the current understanding of its synthesis is based on that of melanin biosynthesis. Studies in our laboratory have demonstrated that the precursor to neuromelanin, dopamine can be enzymatically and non-enzymatically oxidized to form both DNA adducts and oxidative base damage. Based on these observations and the unique association of the presence of neuromelanin and specific neuronal cell loss we propose to determine whether the process of neuromelanin synthesis leads to the production of DNA damage. In order to achieve this goal we propose to: 1a) Optimize the 32P-postlabeling procedure for detection of stable-DNA adducts and HPLC with electrochemical detection for the quantification of both unstable adducts and oxidative base damage formed by dopamine. 1b) Identify the structure(s) of the DNA adducts formed by dopamine using a combination of spectroscopic techniques. 2) Insert the human gene for tyrosine into an expressed plasmid. The expressed protein will be affinity purified and characterized. This human enzyme will be used to study oxidation of dopamine and will provide information as to the enzymatic mechanisms for production of dopamine induced DNA damage. 3) We will engineer PC12 cells to express tyrosine under transcriptional control. These cells, PC12/tyr, will be used to determine if DNA damage occurs during neuromelanin synthesis. Parallel studies with these cells will investigate the induction of cellular cytotoxicity during neuromelanin synthesis. We believe that these Specific Aims will allow us to test our hypothesis that DNA damage can occur during the synthesis of neuromelanin. In addition, the results of these studies will provide unique molecular markers that will be used in future studies to evaluate whether this process is occurring in the substantia nigra of human brain. -

Principal Investigator: BORCHELT, DAVID R
Grant Number: 1R01NS044278-01A2
Title: Protein Misfolding in Neurodegeneration

Abstract: The accumulation of ubiquitin-immuno-reactive material in cell bodies, dendrites, and/or axons of neurons are a prevalent pathology of neurodegenerative disease. It has been suggested that the accumulation of this material is a cellular symptom of reduced ubiquitin/proteasome system (UPS) function. In the present application, we propose four Aims that are designed to probe whether and how the proteasome/ubiquitin system is dysfunctional in various models of neurodegenerative disease. In Aim 1, we propose to use genetic approaches to alter the activities of UPS components. We have been provided mice lacking parkin (a biquitin E3 ligase whose loss triggers Parkinson's disease), and we would like to cross these mice to our APPswe/PS1dE9 mice. We hypothesize that amyloid deposition, in a context of parkin deficiency, may induce novel cytoplasmic pathologies, such as Lewy-body-like inclusions. Aim 2 will develop systems to inhibit proteasome function in vivo in transgenic mice, both chronically and acutely, using genetic approaches. Aim 3 will build on recent characterization of a subset of sporadic ALS cases, where we have identified cystatin C as a protein of interest in the disease. To test the role of this protein in ALS, we propose to create transgenic animals that express elevated levels of the human protein. Aim 4 will focus on identifying the protein backbone constituents of the ubiquitin immunoreactive material that accumulates in our mouse models of Alzheimer's disease and ALS. This Aim will involve the development of transgenic mice expressing recombinant ubiquitin molecules carrying peptide motifs that facilitate detection and purification. Collectively, these studies should allow us to examine the role of proteasome dysfunction in disease pathogenesis and perhaps identify some of the mis-folded proteins that accumulate in disease-associated inclusions. -

Principal Investigator: Botas, Juan
Grant Number: 5R01NS042179-04
Title: Neurodegeneration with Drosophila

Abstract: The ultimate goal of this project is to gain insight into polyglutamine-induced neurodegeneration by identifying genes, pathways and molecular mechanisms involved in the pathogenesis of spinocerebellar ataxia type 1 (SCA1). A Drosophila model of SCA1 was created by generating flies that express either normal or expanded human SCA1 transgenes. This fly model recapitulates the cellular phenotypes observed in SCA1 patients including the formation of nuclear inclusions (NI) and progressive neuronal degeneration. Capitalizing on the power of Drosophila genetics, two large genetic screens were designed to identify genes that modify a SCA1 neurodegenerative phenotype in the eye. The first screen yielded modifiers of the SCA1 phenotype when gene activity was decreased; the second screen yielded SCA1 modifiers when gene activity was increased. Both suppressors and enhancers of the neurodegenerative phenotype were obtained from each screen. The first aim of the proposed work is to identify the genes that modify the SCA1 neurodegenerative phenotype. These modifiers will be further characterized in sensitive viability and locomoter assays that allow the quantification of their modifier effects. The most powerful suppressors will be selected for further studies. To investigate whether different polyglutamine disease share common mechanisms of pathogenesis, the SCA1 modifiers will be tested in fly models of Huntington disease and polyglutamine toxicity. Finally, because the normal function of the SCA1 gene may be relevant to pathogenesis, the function of the Drosophila SCA1 gene will be investigated by generating lack-of-function mutations and transgenes for its over expression. In future studies, the most promising SCA1 suppressors characterized in flies will be investigated in the SCA1 mouse model, and in mouse models of polyglutamine disease. These genes may also be relevant to research aimed at treating other neurodegenerative proteinopathies such as Alzheimer disease and Parkinson disease. They will provide valuable targets for future pharmacological research aimed at developing drugs for therapy. -

Principal Investigator: BREDESEN, DALE E
Grant Number: 5R01NS033376-07
Title: Novel apoptotic pathway activated by misfolded proteins

Abstract: Neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS) and prion protein diseases all feature misfolded proteins and their aggregates, which appear to play a role in disease pathogenesis. However, the mechanism(s) and pathways by which misfolded proteins couple to the cell death program is poorly understood. We have recently found that misfolded proteins, which trigger endoplasmic reticulum stress (ER stress), induce a novel intrinsic apoptotic pathway that is independent of Apaf-1 and mitochondria (Rao et al., 2001; Rao et al., 2002a; Rao et al., 2002b). In order to define the molecular requirements of this pathway, we have developed a cell-free system of ER stress-induced apoptosis. In this system, microsomes isolated from cells lacking ER stress fail to activate cytosolic extracts, whereas microsomes isolated from cells undergoing ER stress activate caspases-3 and -9 in cytosolic extracts. Using a set of complementary approaches including protein purification procedures, 2D MALDI-TOF/nano-ESMS (two-dimensional matrix-assisted laser desorption ionization-time of flight/nanoelectrospray mass spectrometry), immunodepletion of candidate proteins, and an RNA interference (RNAi) approach, we have identified the initial candidate biochemical mediators of this novel apoptotic pathway, and here we propose to continue to identify the components of this ER stress-induced apoptotic pathway. Because an understanding of the relationship between the accumulation of misfolded proteins, cellular stress response, and cell death programs should facilitate the development of new therapeutic strategies for neurodegenerative disorders that feature misfolded proteins, we propose to integrate the findings from our cell work with an animal model of ALS, and address the following questions: (1) What are the proteins that mediate ER stress-induced cell death? (2) Within the set of relevant proteins that are differentially expressed, what are the crucial proteins for cell death induction? (3) Is there evidence of activation of the ER stress-induced apoptotic pathway in transgenic mice expressing mutant (vs. wild type) CuZnSOD? (4) Does recombinant mutant CuZnSOD protein induce normal organelles to initiate a specific cell death pathway? We believe that the results of the proposed experiments may offer insight into the pathogenesis of neurodegenerative disorders that feature misfolded proteins, and should enhance the usefulness of our system for development of therapeutics.-

Principal Investigator: BROADIE, KENDAL S
Grant Number: 5R01NS041740-05
Title: Synaptic Mechnisms in Drosophila Neurodegeneration Model

Abstract: The hypothesis driving this proposal is that presynaptic dysfunction is a common causative factor leading to cell death in multiple inherited neurodegenerative diseases. This hypothesis is based on the observations that 1) synaptic function mediates neuronal survival during development, 2) mutations which strongly impair presynaptic function result in massive, progressive neuronal degeneration, 3) a number of presynaptic proteins have been directly implicated in neurodegenerative diseases and 4) neuronal dysfunction/synapse loss is known to precede by a substantial period the manifestation of cell death in these diseases. To date, however, there is no established direct evidence of synaptic dysfunction mediating neuronal death during neurodegenerative disease states. The goal of this proposal is to assay synaptic maintenance in two genetic models of neurodegenerative diseases: Drosophila models of Parkinson's Disease (PD), a classic "protein storage" disease, and Niemann-Pick Type C (NP-C), a classic "lipid storage" disease. Drosophila was selected for its attractive properties as a new molecular genetic model of neurodegeneration, and its long history as the foremost genetic model for synaptic studies. PD and NP-C were selected as representative of a large number of related neurodegenerative disorders. The Drosophila PD model has been recently established through transgenic over-expression of human alpha-synuclein (a presynaptic protein) and shown to accurately recapitulate the diagnostic features of human PD. A Drosophila model of NP-C is being established through mutation (loss-of-function) of the endogenous NPC I gene, the known cause of human NP-C disease. Specifically, this proposal is to conduct age-progressive studies of synaptic mechanisms in Drosophila PD and NP-C models to correlate synaptic maintenance with the onset, progression and prevalence of neurodegeneration. The first aim is to improve Drosophila models by generating fluorescently tagged alpha-synuclein and NPCI proteins whose levels can be reversibly regulated through a temperature-dependent ubiquination strategy. Secondly, to confirm gross features of neurodegeneration in these models with behavioral assays and examination of nervous system/neuronal architecture. Third, and most importantly, to assay synaptic development, function and maintenance in these models. Assays will include electrophysiological measurements of neurotransmission, quantitative fluorescent optical imaging of protein and lipid dynamics in the presynaptic terminal and ultrastructural studies of presynaptic architecture. Together, these studies will allow a conclusive determination of whether synaptic maintenance is

Principal Investigator: BURKE, ROBERT E
Grant Number: 5R01NS026836-15
Title: Apoptosis in substantia nigra

Abstract: The theme of our investigations has been that the molecular pathways of programmed cell death (PCD) may be relevant to the pathogenesis of Parkinson's disease and allied disorders. In recent years it has also become apparent that PCD regulates viability in cell-based therapeutic approaches, including tissue implants and neural stem cells. There has been tremendous growth in our knowledge of the molecular basis of PCD. However, most of this knowledge derives from relatively simple in vitro systems. While there are universal aspects of PCD mechanisms, it is nevertheless also clear that PCD is context dependent. It is therefore essential to translate this new knowledge to the in vivo context. A unique aspect of our approach is to examine PCD in postmitotic, phenotypically defined dopamine (DA) neurons in living brain. We will investigate two themes related to regulation and effector mechanisms of cell death in these neurons. While there is evidence that natural cell death (NCD) in DA neurons is regulated by striatum-derived neurotrophic support, the nature of these factors remains unknown. Theme 1 will examine the possibility that GDNF or neurturin (NTN) may serve as such factors. Our first Aim will examine the effect of increased striatal expression of GDNF, in a unique temporally-regulated bi-transgenic model, on the mature number of DA neurons. The second Aim will determine whether endogenous striatal GDNF regulates the magnitude of NCD in DA neurons, through the application of "knock down" approaches. Our third Aim will directly compare the potency of GDNF and NTN to suppress apoptotic death in a developmental axotomy model. Theme 2 will seek to identify important proteases mediating PCD in DA neurons in vivo. We have shown that activated caspase-3 is expressed in apoptotic DA neurons. In Aim IV, we will examine the functional significance of its expression, by studying the magnitude and protein cleavage characteristics of NCD, and the size of DA progenitor pools, in caspase-3 null animals. Many in vitro studies have shown that caspases-independent pathways exist. In Aim V, we will examine expression of the proteasome complex in PCD in DA neurons in vivo. The new knowledge gained by the studies outlined in this application will have direct implications for concepts of pathogenesis of Parkinson's disease and for approaches to optimizing cell-based treatments. -

Principal Investigator: BURKE, ROBERT E
Grant Number: 2P50NS038370-06
Title: Mechanisms of dopamine neuron degeneration

Abstract: Parkinson's disease (PD) is a prevalent and disabling neurological disease characterized by the progressive loss of motor control due to the degeneration of dopamine (DA) neurons of the substantia nigra. Among neurodegenerative diseases, PD has served as a model for the development of novel therapeutic approaches: administration of neurotransmitter precursors (levodopa), cell implantation, and more recently, deep brain stimulation. As important and effective as these advances have been, they only relieve symptoms; none stop the progression of the disease. In order to develop therapies which halt the progression of the disease, we need to achieve a better understanding of the pathogenesis of DA neuron degeneration. This submission represents a competing continuation application for a Morris K. Udall Parkinson's Disease Research Center of Excellence awarded to Columbia University in 1999. This renewal consists of four projects devoted to a single integrating theme: to understand the molecular and cellular mechanisms of dopamine neuron degeneration. While there are many worthy hypotheses of pathogenesis, the subprojects of this proposal will focus on four major current themes in the pathogenesis of PD, related to the roles of: (1) Abnormal intracellular protein degradation; (2) Inflammatory pathways; (3) Programmed cell death (PCD); and (4) Oxidative injury. In Project 1, Dr Serge Przedborski will evaluate the role of cyclooxygenase 2 (COX2) and cytosolic phospholipase A2 (cPLA2) (Theme 2) in mediating dopamine neuron damage in the MPTP model of PD and in human brain samples. In Project 2, Dr David Sulzer will examine in astrocyte and neuron primary cultures the role of chaperone mediated autophagy in the degradation of proteins implicated in PD (Theme 1) and the effect of these proteins on catecholamine sequestration (Theme 4). In Project 3, Dr Robert Burke will use genetic techniques in animal models to examine the roles of the mixed lineage kinases, Akt and JNK in mediating PCD in dopamine neurons (Theme 3), and he will evaluate the functional role of ER stress in initiating cell death (Theme 1). In Project 4, Dr Lloyd Greene will continue to evaluate the functional role of genes identified in the current funding period by SAGE analysis as upregulated following neurotoxin exposure. He will continue his studies of the role of ER stress-related genes (Theme 1) and genes implicated in PCD (Theme 3) in PC12 cells and primary sympathetic neurons, and in living animal models (the latter in collaboration with Drs Burke and Przedborski). He will also examine these transcripts and their protein products in PD brain. -

Principal Investigator: Caron, Marc G.
Grant Number: 2R01NS019576-21
Title: Dopamine Receptors: Characterization and Regulation

Abstract: The G protein-coupled receptors (PCRs) comprise a broad family of receptors that activate a large number of effectors in response to a variety of signals including amines, photons, lipids, peptides and proteases. Signaling through CPCRs requires the coordinated balance between proceses that govern receptor activation, desensitization and resensitization. Desensitization of GPCRs involves receptor phosphorylation by specific G protein-coupled receptor kinases (GRKs) and interaction with arrestin proteins. GRKs and arrestins play an important role not only to desensitize second messenger signaling but also contribute to the endocytosis of GPCRs and their ability to recycle back to the plama membrane and resensitize. In addition, the complex of receptor/arrestin engages a variety of signaling pathways including those associated with Src family kinases and components of the MAP kinase cascades. The overall objective of the proposed research is to define the basic molecular and cellular mechanisms that contribute to these processes with the goal of better understanding physiological and pathological conditions. The dopamine and adrenergic receptors will be used as prototypic GPCRs for many of the proposed studies. Aim 1: We will attempt to identify the molecular determinants by which the specificity of action of GRKs and arrestins is established. In addition, we will examine how constitutive phosphorylation and interaction of GPCRs with arrestin can underlie the loss-of-function phenotype of certain mutant GPCRs both in cellular and in vivo systems. Aim 2: The molecular basis of the ability of arrestins to act as endocytic switch and control the resensitization process of GPCRs will be examined. Aim 3: How the pardigm of GRKs and arrestins might apply to non-conventional GPCRS such as frizzled and smoothened will also be examined both in cellular and in vivo model systems. Aim 4: These experiments will explore the hypothesis that the behavioral and biochemical synergisms that exist between dopamine D 1 and D2 receptors results from the oligomerization of these receptors. Results from these studies should broaden our understanding of the role of GPCRs and their regulation in normal physiology and disease states and provide insight into potential new therapeutic approaches for conditions such as heart failure, drug abuse, and psychiatric disorders.-

Principal Investigator: CHAO, MOSES V
Grant Number: 2R01NS021072-19
Title: Molecular Analysis of Nerve Growth Factor Action

Abstract: Neurotrophins represent an important family of polypeptide growth factors which influence the proliferation, differentiation, survival and death of neuronal and non-neuronal cells during vertebrate development They have been proposed as therapeutic agents for neurodegenerative disorders and nerve injury However, clinical applications have met with very disappointing results, in part due to difficulties of delivery and pharmacokinetics in the nervous system and unanticipated side effects We have found a way to use small molecule ligands of G protein-coupled receptors (GPCR) to activate Trk receptors in the absence of neurotrophin binding These small molecules keep neurons alive by stimulating the actions of trophic factor receptors Ligands for G protein-coupled receptors represent a novel way of stimulating neurotrophin receptor signaling, however, the mechanism of this process is unknown This grant will investigate the cell biological mechanisms that account for transactivation of neurotrophin receptors in neurons and define the contribution of receptor trafficking and transport to this process Defining the proteins that regulate neurotrophin receptor internalization, translocation and signaling is critical to our understanding of normal neuronal development and function as well as perturbations that occur in response to injury or disease. Our findings are directly relevant to the understanding and treatment of neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases and amyotrophic lateral sclerosis.-

Principal Investigator: CHEN, JIANG F
Grant Number: 5R01NS041083-05
Title: NOVEL BENEFIT OF A2A RECEPTOR INACTIVATION IN PD MODELS

Abstract: (Adapted from the Applicant's Abstract) Parkinson's disease patients experience profound depletion of striatal dopamine (DA) due to degeneration of the nigrostriatal DA pathway. The predominant treatment for the past 30 years has been the DA precursor, L-dopa. While this strategy improves motor deficits, it has no effect on the underlying degenerative process, and indeed can have the additional unwanted side-effect of inducing dyskinesia. A possible alternative therapy, with neuroprotective ability appears to be use of antagonists of a specific class of adenosine receptors, A2A. These agents appear to have both motor-activating properties and preliminary data suggest they may also attenuate MPTP-induced DA neurotoxicity and prevent the locomotor stimulation that occurs with chronic DA receptor stimulation. The proposed studies will systematically investigate the novel motor and neuroprotective effects of A2A receptor antagonists. Methods center around pharmacological studies and use of genetic knockout (KO) approaches. There are three specific aims: 1) to test the hypothesis that A2A inactivation enhances motor function through D2R-dependent and independent mechanisms using A2AR-KO, D2R-KO and double KO mice; 2) to test the hypothesis that A2AR inactivation prevents the development of chronic L-dopa-induced rotational motor sensitization in unilateral 6-OHDA-lesioned mice; and 3) to characterize the role of V in MPTP-induced neurotoxicity by establishing the potency, "therapeutic window" and by "analyzing synergy between A2AR activation and inactivation;" in addition, the effect of A2AR agents on MPTP metabolism in vivo and in cell culture will also be examined to investigate the neurochemical mechanisms of protection by A2AR inactivation. -

Principal Investigator: CHEN, JUN
Grant Number: 5R01NS044178-02
Title: Apoptosis Execution Pathways in Dopaminergic Cell Death

Abstract: Parkinson's disease (PD) is characterized by progressive and selective loss of dopaminergic neurons in substantial nigra pars compacta. Although the etiology remains unknown, there is considerable evidence that supports the view that programmed cell death (PCD) contributes, at least in part, to the degeneration of dopaminergic neurons in PD. Recent studies have identified a group of terminal caspases, particularly caspase-3/-7, as the central executive molecules in neuronal PCD. Caspase-3 actively participates in dopaminergic neuronal cell death in response to PD-relevant insults, although how caspase-3 is activated in this process is largely elusive. Furthermore, caspase-3 and other terminal caspases may not be the only executioners of neuronal PCD. A novel pro-apoptotic molecule, designated as AIF (apoptosis-inducing factor), has now been identified. AIF, which is activated and released from the mitochondria upon receiving death signals, and potently promotes high-molecular-weight DNA fragmentation and nuclear apoptosis. Bcl-2 family proteins are important PCD regulators and have been implicated in dopaminergic neuronal cell death. The pro-apoptosis Bcl-2 family member Bax is a well-characterized cell death effector, which, upon activation, targets mitochondria and triggers the cytochrome c-dependent intrinsic pathway. However, whether Bax activates AIF and the mechanism by which Bax is activated in dopaminergic neuronal apoptosis is unknown. We have now obtained exciting preliminary data which suggest that 1) caspase-3 activation in dopaminergic neurons is dependent on the intrinsic pathway; 2) both AIF-dependent and caspase-dependent mechanisms may contribute independently and synergistically to the final execution of dopaminergic neuronal apoptosis; 3) Bax is a direct mediator of AIF release in neurons, and the activation of Bax during dopaminergic cell death appears to be p53-dependent; and 4) Bak may be an important cofactor that enhances the pro-apoptotic effect of Bax at the mitochondria. Therefore, the objective of this project is to determine the role of caspase-3-dependent and AIF-dependent death execution pathways in dopaminergic neuronal cell death following PD-relevant insults and to determine the role of Bax in triggering the activation of these two pathways. The proposed studies will be performed using complementary in vitro and in vivo model systems, and will take advantage of our recent cloning of novel dominant-negative inhibitory mutants of caspase-9, Apaf-1 and AIF, and the availability of Bax-deficient mice. The following specific aims will be addressed: 1. Test the hypothesis that the caspase-9/Apaf-1 intrinsic pathway plays a central role in

Principal Investigator: Chu, Charleen T
Grant Number: 5R01NS040817-04
Title: THE PARKINSONIAN 6 HYDROXYDOPAMINE MODEL

Abstract: Parkinson's disease is the most common debilitating movement disorder of the aging human population. The neurons that degenerate in Parkinson's disease are subject to increased oxidative stress because superoxide and other reactive species are generated during dopamine metabolism. 6-hydroxydopamine (6-OHDA) is a redox cycling dopamine analog, which can be targeted to selectively damage the nigrostriatal system that degenerates in Parkinson's disease. Phosphotyrosine signaling pathways activated by neuroprotective factors, such as brain derived neurotrophic factor and glial cell line-derived neurotrophic factor, are important for dopaminergic neuron function and survival. This proposal is designed to investigate the hypothesis that oxidant-mediated alterations in phosphotyrosine signaling contribute to degeneration of dopaminergic neurons in Parkinson's disease. Nitrotyrosine, a marker of oxidative stress involving peroxynitrite formation, is increased in both the 6-OHDA rodent model and in human Parkinsonian brain tissues. Peroxynitrite is formed from the reaction of superoxide with nitric oxide, implicating these free radicals in the pathogenesis of Parkinson's disease. In this proposal, mechanisms by which 6-OHDA, superoxide, and nitric oxide affect phosphotyrosine signaling cascades will be investigated using immortalized dopaminergic neuron lines and mice with genetically altered levels of extracellular superoxide dismutase. This comprehensive set of studies will yield important insights concerning mechanisms by which oxidative stress affects neurotrophic signaling in dopaminergic neurons, potentially contributing to development of combined antioxidant-neurotrophic factor therapies for Parkinson's disease. -

Principal Investigator: COLLIER, TIMOTHY J.
Grant Number: 5R01NS042125-04
Title: Cell Grafts for Parkinson's Disease

Abstract: In vitro expansion of neural progenitor cells followed by induction of dopaminergic phenotype may provide a limitless source of cells for grafting into patients with Parkinson's disease (PD). However, the signals controlling the conversion of these cells into dopamine (DA) neurons must be identified. In an effort to accomplish this, single cells isolated from ventral mesencephalon were clonally expanded and exposed to hematopoeitic cytokines and neurotrophic molecules. Analysis of cell differentiation in response to this treatment yielded conversion of a high percentage (72 to 98 percent) of cells in some clones to a tyrosine hydroxylase (TH)-positive phenotype. Of the 24 clones generated, the best conversion to TH cells occurred with exposure to a combination of interleukin-1 (IL-1), interleukin- 11 (IL-11), leukemia inhibitory factor (LIF), and glial cell line-derived neurotrophic factor (GDNF). Positive clones expressed TH, the DA transporter, Nurr-1 and released DA in culture. Other cells in cytokine-exposed clones expressed GFAP (astrocyte marker) or MAP-2 (neuron marker) indicating that the original neurospheres were also capable of producing clones that differentiate into glial and nondopaminergic neurons. Initial neural grafting studies m the rat model of PD using a clone with the highest conversion rate to TH indicated that converted progenitor cell grafts produced complete amelioration of amphetamine-induced rotational behavior and continued to express the TH phenotype. However, the survival rate of these grafted progenitor cells was reduced (26 percent) compared to embryonic ventral mesencephalon (VM). The experiments proposed here will develop protocols for optimal survival of Wafted cytokine-converted mesencephalic progenitor cells. Once survival of grafted mesencephalic progenitor cells is optimized, direct comparisons will be made to fresh embryonic VM grafts on measures of behavior, in vivo dialysis, post-mortem DA biochemistry, DA receptors, cell survival and neurite extension. Lastly, this proposal will test the efficacy of the DA conversion cocktail on clonal progenitors derived from embryonic mesencephalon of nonhuman primate brain. If successful, cytokine-converted mesencephalic progenitor cells could potentially replace embryonic tissue as the primary source of cells for grafting in PD. -

Principal Investigator: COLMAN, DAVID R
Grant Number: 5R01NS041687-04
Title: Protein Components of the Synaptic Adhesive Scaffold

Abstract: In the most contemporary view, the central nervous System synapse may be thought of as comprising 2 discrete subdomains which overlap structurally and functionally. The first domain is the synaptic "scaffold" which is observed by electron microscopy, consisting of apposed, rigorously parallel presynaptic and postsynaptic plasma membrane thickenings bound together by "crossbridges" that span the synaptic cleft. The scaffold is retained even after vigorous fractionation and detergent extraction of synaptosomes, and it seems clear that it is held together by adhesion molecules, whose identities remain unknown at present. The second subdomain is the neurotransmissional machinery through which the synapse mediates its primary physiological functions. This subdomain is superimposed upon the scaffold and interacts with it via molecular forces we don't understand as yet. Much effort has been focused on analyzing the physiological components of the synapse; however, the major constituents of the scaffold and how its intercellular adhesive components interact have remained elusive. This is because of inadequate fractionation techniques for the purification of intact CNS synaptic junctions, and the bewildering array of candidate proteins that may operate at different synapses. It is clear that efforts to recognize and evaluate changes in synaptic proteins which occur during degenerative processes must rely first on a complete catalog of the structural proteins involved in synaptic development and maintenance and how they interact with each other; and second, on an understanding of the intracellular binding partners for these scaffolding molecules which function in synaptic signaling phenomena, and in attachment to the underlying subsynaptic components. Long range, we want to understand exactly how molecular adhesive forces organize and stabilize the pre-to post-synaptic scaffold of the synaptic junctional complex in the CNS. We propose to: I) use a novel cell fractionation procedure we devised to purify synaptic junctional complexes in high yield, and then use newly developed methods in mass spectrometry to identify the component adhesion and adhesion associated molecules, and II) begin studies on the interactions between these molecules which lead to the assembly of the synaptic junctional complex in the CNS. -

Principal Investigator: COOPER, DERMOT M
Grant Number: 5R01NS028389-11
Title: INTRACELLULAR CALCIUM CONTROL OF cAMP SYNTHESIS

Abstract: Adenylyl cyclases generate the ubiquitous second messenger cAMP. The importance of this messenger in regulating a large number of cellular processes is acknowledged. However, the methods for measuring cAMP lack both temporal and spatial resolution, leading to the widespread belief that these signals are simple. Against this notion, the large number of interactions between signaling pathways for Ca2+ and cAMP have been demonstrated and the dynamic nature of Ca2+ makes it likely that cAMP signals are also complex. In particular, Ca2+-sensitive adenylyl cyclases are regulated discreetly by physiological modes of Ca2+ entry and cAMP modulates Ca2+ entry by a variety of mechanisms. The present application proposes to develop adenylyl cyclase/ aequorin and adenylyl cyclase/ cameleon chimeras as localized sensors of Ca2+ and to develop olfactory cyclic nucleotide gated (oCNG) channels as rapid membrane-bound sensors of cAMP. Once these sensors are optimized, the applicant intends to examine dynamic and interdependent changes in the two signals in pituitary-derived GH4 cells, an excitable cell type. He will also use the cAMP sensor to study the rapid kinetics of adenylyl cyclase regulation in its native environment. These studies represent a first attempt to associate cAMP and Ca2+ signaling on the same temporal and spatial scale. In the long-term, such information will lead to greater generalized understanding of how the coordinated activity of these two second messengers control cellular function. -

Principal Investigator: CRAIGEN, WILLIAM J.
Grant Number: 5R01NS042319-04
Title: The Role of Mitochondrial VDACs in Apoptosis

Abstract: Apoptosis is a form of directed cellular death that is essential for a variety of biological processes, including embryonic development, cancer surveillance, and host defense. Inappropriate apoptosis occurs in autoimmune disorders, malignancy, and acquired and heritable neurodegenerative disease. Two parallel pathways for apoptosis have been uncovered, one mediated by the release of cytochrome c from the mitochondrial intermembrane space, and a second that bypasses cytochrome c release by directly activating caspase 8. The release mechanism for cytochrome c remains controversial, with evidence suggesting that the mitochondrial outer membrane channels; VDACs, open to conduct cytochrome c. In mammals there exist three VDAC isoforms. The laboratory has generated cell lines and mice that are deficient for each VDAC or a subset of VDACs. It is proposed to use these cell lines and mice to define the role VDACs play in cytochrome c mediated apoptosis. Specifically, the kinetics and extent of apoptosis will be determined in the deficient cell lines and mice. Cytochrome c release will be directly quantified and binding of pro and anti-apoptotic protein factors to mutant mitochondria will be measured. Expression profiles following induction of apoptosis will be performed using microarrayed cDNAs. Finally, point mutations will be introduced into VDACs that will abrogate voltage dependent channel closure to determine if open channels interfere with apoptosis. These studies may validate VDACs as targets for inhibiting or enhancing apoptosis. -

Principal Investigator: CROW, JOHN P
Grant Number: 5R01NS040819-04
Title: Mechanism of Selective Toxicity of SOD1 Mutants in ALS

Abstract: In 1993, it was first reported that a mutation to the gene coding for Cu,Zn superoxide dismutase (SOD1) was associated with a form of familial amyotrophic lateral sclerosis (ALS). Since that time, more than 70 single amino acid mutations to SOD1 have been found to cause ALS in humans. Mice transgenic for any one of several of the human SOD1 mutants develop progressive and ultimately lethal paralysis in a time-dependent manner reminiscent of human ALS. Studies in transgenic mice clearly indicate that SOD1 is toxic via a gained function because the mutant produces disease even in the presence of marked increases in total SOD1 enzyme activity. Despite the overwhelming evidence for a gained toxic function, the exact nature of that function has remained elusive. Equally puzzling has been the fact that SOD1 mutants are toxic only to motor neurons even though they are expressed in all cell types. Based on published results, in vitro characterizations of SOD1 mutants, studies in cultured motor neurons, and preliminary data from transgenic mice, we have formulated a hypothesis which may explain how all ALS-associated SOD1 mutations can be toxic via a common mechanism and why toxicity is manifested only in motor neurons. HYPOTHESIS: We are proposing that zinc-deficient (copper-containing) SOD1 is the common toxic phenotype of ALS-associated SOD1 mutants, that zinc-deficient SOD1 is injurious via its ability to utilize ascorbate, oxygen, and nitric oxide to catalyze the formation of the cytotoxin peroxynitrite, and that neurofilament proteins--which avidly bind zinc and are very abundant in motor neurons--contribute to the formation of zinc-deficient SOD1 preferentially in motor neurons. This study proposes: Specific Aim 1) to measure zinc-deficient SOD1 in the most widely used animal model of ALS (G93A transgenic mice) and determine the factors responsible for its accumulation, Specific Aim 2) to determine the conditions which lead to SOD1-mediated oxidant generation and its potential relationship to toxic protein aggregation, and Specific Aim 3) to evaluate the in vivo efficacy of two classes of compounds which protect cultured motor neurons from the toxic effects of zinc-deficient SOD1 and which enhance survival in G93A mice. -

Principal Investigator: DAUER, WILLIAM T
Grant Number: 1K02NS045798-01A1
Title: The mechanism of MPTP resistance in synuclein null mice.

Abstract: My long-held career goal is to investigate questions of importance to both patient care and fundamental biology. During medical training, I developed a strong interest the basic pathogenic mechanisms of Parkinson's disease (PD), an illness characterized by degeneration of substantia nigra dopamine (DA) neurons and cytoplasmic aggregates of alpha-synuclein (SYN). I came to appreciate the power of genetically modified animals as tools to explore basic aspects of disease pathogenesis, and developed expertise in the generation of such animals. However, I now need to acquire skills necessary to assess the consequences of PD-related mutations on cellular and behavioral aspects of dopaminergic function in these animals. To accomplish this goal, I have developed collaborations with experts in PD research, and will pursue the proposed work within the integrated PD research group at Columbia University. Rarely, PD may be caused by missense mutations in SYN. However, normal SYN function and the mechanism by which pathogenic mutations disrupt SYN biology and lead to PD are poorly understood. MPTP-induced degeneration of DA neurons is a commonly studied model of PD. We find that SYN null mice display striking resistance to MPTP-induced degeneration of DA neurons, and this resistance appears to result from an inability of the toxin to access and inhibit its target, mitochondrial complex I. The goal of this research plan is to exploit this robust phenotype of SYN null mice to gain insight into the normal function of SYN, and explore how this function is altered by PD-causing mutations. In Aim 1 we will measure whether known concomitants of complex I inhibition (increased lactate and reactive oxygen species; decreased ATP) are also impaired in SYN null mice, and characterize processes that control access of the toxin to complex I (vesicular and monoamine transporter function). In Aim 2 we will further explore whether altered synaptic function underlies the MTPT resistance of SYN null mice by testing whether they are selectively resistant to toxins that traffic through the synapse. In Aim 3, by restoring wild type or mutant SYN to specific neuronal populations of SYN null mice, we will test whether the MPTP resistance is a cell autonomous phenomenon and whether pathogenic SYN mutations modify an aspect of its function involved in effecting MPTP-induced neurodegeneration. This proposal exemplifies the type of clinically related fundamental neurobiological research I plan to pursue during my career.-

Principal Investigator: DAWSON, TED M
Grant Number: 1R21NS047565-01
Title: Models of Familial Parkinson's Disease: DJ-1 Knockouts

Abstract: Mutations in the DJ-1 gene are a rare genetic cause of autosomal recessive Parkinson's disease (PD). The DJ-1 protein is either absent or appears to be functionally inactive in the families in which mutation have been identified. Thus, mutations in the DJ-1 gene probably cause PD through a loss of function. It is difficult at this juncture to fully appreciate how mutations in the DJ-1 gene cause PD, as its function is largely unknown. DJ-1 was identified as a hydroperoxide-responsive protein that becomes more acidic following oxidative stress suggesting that it may function as an antioxidant protein. Furthermore, DJ-1 is sumoylated through binding to the SUMO-1 ligase, PIAS, suggesting that it might be involved in the regulation of transcription. Other putative functions of DJ-1 have been raised, but how a loss of function of DJ-1 leads to loss of DA neurons and PD awaits further study. We propose to generate and characterize DJ-1 knockout mice to formally test the hypothesis that the absence of DJ-1 function is the cause of PD due to DJ-1 mutations. Accordingly experiments are proposed to further characterize the role of DJ-1 in the pathogenesis of PD. In Specific Aim #1 we will develop and characterize DJ-1 knockout mice. In Specific Aim #2 we will evaluate the sensitivity of DJ-1 knockouts to environmental toxins including MPTP-induced dopaminergic cell death. In Specific Aim #3 we will determine whether DJ-1 interacts with parkin by evaluating the effect of crossing DJ-1 knockout mice with parkin knockout mice. Development and characterization of DJ-1 knockouts, understanding the relationship of DJ-1 and parkin in the pathogenesis of PD may provide insight into the molecular mechanisms by which these gene products induce neuronal damage and may provide novel therapeutics and targets to prevent the toxic effects of these familial associated genes in the degenerative process of PD. -

Principal Investigator: DAWSON, TED M
Grant Number: 2P50NS038377-06A1
Title: Parkinson's Disease Research Center of Excellence

Abstract: The overall goals of this proposal are to understand the role of alpha-synuclein, parkin, DJ-1 and synphilin-1 in the pathogenesis and pathology of Parkinson's disease (PD) and to define the molecular mechanisms of neuronal injury in animal models of PD. The program represents a multi-disciplinary, mechanistic approach involving interactive, productive investigators with complementary areas of expertise who have long been committed to the studies of neurodegenerative diseases. Their aim will be to integrate the activities of various disciplines such that the interrelationships will result in a greater scientific contributions and achievements if each project were pursued individually. The program has one major theme: To understand the role of familial associated genes alpha-synuclein, parkin and DJ-1 in the pathogenesis of Parkinson's disease and related disorders. The role of alpha-synuclein, parkin, DJ-1 and synphilin- 1 in PD pathogenesis will be investigated using molecular, transgenic, neuropathologic, cell biologic and neurobehavioral approaches to examine the mechanism of neuronal dysfunction and injury clue to alterations in these gene products. The mechanism of neuronal loss in Parkin knockout mice and alpha-synuclein A53T transgenic mice will be characterized. We will determine whether parkin interacts with alpha-synuclein and further explore the relation between and parkin, alpha-synuclein and synphilin-1. We will explore alpha-synuclein processing and modifications and the relationship of synphilin-1 to alpha-synuclein. Furthermore, we will investigate the potential function of DJ-1 and it role in PD Pathogenesis. We believe that our multi-disciplinary approach has the capacity to produce unique information concerning the mechanisms of neurodegeneration in genetic animal models of Parkinson's disease and the related synucleinopathies and to lead to better understanding of the function and the role of alpha-synuclein, parkin, DJ-1 and synphilin-1 in normal and pathophysiologic processes related to PD. The program consists of four projects: 1) Mouse Models of Parkin Biology and Pathobiology 2) PD Cell Models: Alpha-synuclein and Interacting Proteins; 3) Mechanisms of Neurodegeneration in Human Alpha-synuclein Transgenic Mice; 4) The Role of DJ-1 in Parkinson's Disease and four cores A) Administration and Training; B) Transgenic and Neurobehavior; C) Neuropathology and D) Clinical.-

Principal Investigator: DAWSON, TED M
Grant Number: 1R01NS048206-01
Title: The Role of Parkin in Parkinson's Disease

Abstract: Mutations in the parkin gene are the main genetic cause of autosomal recessive Parkinson's disease (PD) and mutations in parkin also play a major role in familial Parkinson's disease. Preliminary studies indicate a potential pivotal role for parkin in the ubiquitin proteasomal pathway (UPP) by functioning as an ubiquitin E3 ligase. Most disease causing mutations of parkin are thought to be loss of function mutations that ultimately lead to the absence of ubiquitination and the subsequent failure of UPP-mediated degradation of parkin substrates. Thus, the abnormal accumulation of parkin substrates is thought to play a role in the demise of substantia nigra dopaminergic neurons in patients with parkin mutations. A number of putative parkin substrates have been identified, but their importance in the pathogenesis of PD due to parkin mutations is not known. We propose to generate and characterize parkin knockout mice to formally test the hypothesis that the absence of parkin function is the cause of PD due to parkin mutations. Furthermore, biochemical and proteomic characterization of the parkin knockout mice may shed light on the substrates that are important in the pathogenesis of PD due to parkin mutations. Accordingly experiments are proposed to further characterize the role of parkin and it's substrates in the pathogenesis of Parkinson's disease. In Specific Aim #1 we will develop and characterize parkin knockout mice. In Specific Aim #2 we will evaluate the sensitivity of parkin knockouts to environmental toxins including MPTP-induced dopaminergic cell death. In Specific Aim #3 we will evaluate the interaction of parkin with the alpha-synuclein interacting protein, synphilin-1 and determine whether parkin mediates K48 or K63 ubiquitin linkages. In Specific Aim #4 we will determine whether parkin interacts with alpha-synuclein and evaluate the effect of crossing parkin knockout mice with A53T mutant alpha-synuclein transgenic mice. In Specific Aim #5 we will identify and characterize parkin interacting proteins in parkin knockout mice. Development and characterization of parkin knockout mice, understanding the relationship of parkin, alphasynuclein and synphilin-1 in the pathogenesis of PD may provide insight into the molecular mechanisms by which these gene products induce neuronal damage and may provide novel therapeutics and targets to prevent the toxic effects of this familial associated genes in the degenerative process of Parkinson's disease. -

Principal Investigator: DAWSON, VALINA L.
Grant Number: 5R01NS040809-04
Title: Mechanisms of Ischemic Tolerance

Abstract: The overall goal of this project is to understand the molecular mechanisms of ischemic tolerance in cortical neurons. Neuronal ischemic preconditioning or tolerance is a phenomenon in which brief episodes of ischemia protect against the lethal effects of subsequent periods of prolonged ischemia. The signaling mechanisms leading to preconditioning are poorly understood but have the potential for providing important pharmaceutical targets for the treatment of patients at risk for ischemic injury and possibly the treatment of patients suffering from chronic neurodegenerative diseases such as Parkinson's Disease. Ischemia can be modeled in vitro by oxygen-glucose deprivation (OGD). We have recently discovered that OGD preconditioning induces p2 p21ras (Ras) activation in a NMDA receptor- and NO-dependent manner. OGD preconditioning is dependent on Ras activation of the Raf-Mek-Erk pathway. Our observations indicate that activation of the Ras/Erk cascade by NO is a critical mechanism for the development of OGD tolerance in cortical neurons, which may also play an important role in ischemic preconditioning in vivo. To further our understanding of preconditioning it is essential to identify the transcriptional elements that are activated and the new proteins that are responsible for this remarkable neuroprotection. In this project we propose to investigate the role of transcriptional targets of the Ras/Erk signaling cascade with a focus on CREB and Elk activation. We will identify genes that are regulated by preconditioning and determine which genetic changes are responsible for preconditioning. Preconditioning can also be induced by potassium depolarization in an in vitro model of spreading depression. We will investigate whether similar or different mechanisms are responsible for potassium depolarization induced tolerance. We anticipate that this series of investigations will identify endogenous protective mechanisms that ultimately may be harnessed as novel protective strategies against ischemic and traumatic injury as well as chronic neurodegenerative disorders such as Parkinson's Disease. -

Principal Investigator: DEBBURMAN, SHUBHIK
Grant Number: 1R15NS048508-01
Title: Yeast Model for Two Neurodegeneration-Linked Proteins

Abstract: Budding Yeast (S. cerevisiae) has emerged as a powerful model system for understanding molecular aspects of many human diseases. Protein misfolding linked to certain neurodegenerative diseases (NDDs) like Huntington Disease, Lou Gehrig's disease, and prion diseases have been successfully recapitulated in S. cerevisiae and led to identification of therapeutically relevant regulators of misfolding. No S. cerevisiae models for Parkinson's Disease (PD) or dentatorubral pallidoluysian atrophy (DRPLA) have been reported. PD is one of the most common NDDs, while DRPLA is a rare inherited NDD of the triplet repeat disease family. In both diseases, misfolding of a specific protein (alpha-synuclein for PD and atrophin for DRPLA) is thought to cause selective neuronal death. Unlike the well-characterized huntingtin protein in Huntington Disease (which shares many similarities to DRPLA), less is known about the misfolding of mutant atrophin in DRPLA. A S. cerevisiae expression system for studying alpha-synuclein has recently been developed in our lab. Preliminary evidence supports that both wildtype and disease-associated mutants are aggregating within yeast cells and upon purification. A similar effort to establish atrophin-1 expression in yeast is underway. To extend initial observations with alpha-synuclein in yeast and fully develop a yeast model for atrophin, three goals are proposed. 1) Misfolding properties between wildtype and mutant versions of both proteins will be investigated in vivo (immunofluorescence and GFP-based localization and assessment of protein half-life) and in vitro (by measuring protease sensitivity and differential solubility). 2) Influences of chaperones and ubiquitin-proteasomal pathway proteins on folding and degradation of these proteins will be assessed in strains compromised for chaperone/proteasomal function, or those that overexpress chaperones, and by co-immunoprecipitation assessment. 3) A fission yeast (S. pombe) expression model for alpha-synuclein and atrophin properties (as in Aim 1) will be developed and compared with the S. cerevisiae model; NDD models have not been reported in S. pombe. These studies may further clarify the molecular bases for misfolding and degradation of PD- and DRPLA-linked proteins and extend the usefulness of yeast models. Importantly, the scientific training of many undergraduates will be supported, strengthening their cell biology and molecular genetics skills and appreciation for model organisms. -

Principal Investigator: DEBBURMAN, SHUBHIK
Grant Number: 3R15NS048508-01S1
Title: Yeast Model for Two Neurodegeneration-Linked Proteins

Abstract: Unavailable

Principal Investigator: DEFRANCO, DONALD B
Grant Number: 5R01NS038319-05
Title: OXIDATIVE STRESS AND NEURODEGENERATION

Abstract: The Hsp90-binding benzoquinoid ansamycin, geldanamycin (GA) protects Ht22 cells and immature primary rat cortical neuron cultures from glutamate induced oxidative toxicity. Furthermore, preliminary results suggest that GA given during resuscitation improves neurological outcome in rats subjected to global ischemia induced by asphyxial cardiac arrest. GA binding to HSP90 disrupts various intracellular signaling pathways and leads to induction of Hsp70, depletion of the Raf-1 protooconcogene, and reduced activation of downstream targets of Raf-1, ERK-1 and ERK-2. The downregulation of ERK activation caused by GA treatment might be an important component of its neuroprotective activity since inhibition of an ERK activating kinases (i.e. MEK-1) also protects against oxidative toxicity in Ht22 cells and primary rat cortical neuron cultures. We hypothesize that manipulation of Hsp90 function may be a useful strategy to impact various signal transduction pathways in vivo that trigger neuronal cell death. The identification of the molecular mechanisms involved in neuroprotection associated with pharmacological manipulation of Hsp90 function is the major goal of this application. In specific aim 1 we will determine the impact of Hsp90 regulated signaling pathway on glutamate-induced oxidative toxicity in the Ht22 mouse hippocampal cell line and address the following questions. Does Hsp70 induction contribute to the protective effects of Hsp90 binding drugs in vitro? Is persistent ERK activation necessary and sufficient for glutamate-induced oxidative toxicity in Ht22 cells? Does glutamate-induced oxidative toxicity affect other members of the MAPK family (i.e. JNK/SAPK and P38MAPK)? In specific aim 2 we will determine the impact of Hsp90-regulated signaling pathways on glutamate-induced oxidative toxicity in immature primary rat cortical neuron cell cultures. Are GA and U1026 protective against oxidative toxicity in immature primary rat cortical neuron cell cultures? What biochemical events are associated with neuroprotective effects of Hsp90-binding drugs in immature primary rat cortical neuron cultures? Finally in Specific Aim 3 we will determine whether Hsp90 binding drugs are effective post-treatment neuroprotective agents in rat models of global ischemia. Do Hsp90-binding drugs improve neurological outcome following asphyxial cardiac arrest or middle cerebral artery occlusion? Do Hsp90-binding drugs induce Hsp70 in vivo? Do Hsp90-binding drugs affect MAPK activation in vivo? -

Principal Investigator: DENG, HAN-XIANG
Grant Number: 5R01NS040308-04
Title: TRANSGENIC STUDIES OF AMYOTROPHIC LATERAL SCLEROSIS

Abstract: The goal of this project is to investigate the pathogenic mechanisms underlying the neurodegeneration of amyotrophic lateral sclerosis (ALS) using transgenic mouse models. Our application has two immediate Aims: The first one is to replace the two free cysteine residues in mutant human SOD 1 protein to test the role of free -SH groups in the formation of intracellular aggregates noted in ALS. The second aim is to define the smallest fragment of SOD 1 that would still cause ALS in transgenic mice. AIM 1: About 20 percent of the familial ALS cases are caused by mutations in Cu/Zn superoxide dismutase gene (SOD 1). Transgenic mice that over express mutant SOD 1 develop an ALS-like phenotype and motor neuron degeneration. A common feature in the pathology of both human SOD1-linked ALS and the ALS (SOD1) mouse models is the presence of the SOD 1-inimunoreactive inclusions or aggregates in neurons of the brain and spinal cord. These inclusions/aggregates are thought to be important elements in motor neuron death in ALS. To test the hypothesis that these inclusions/aggregates may be formed by disulfide bonds through interaction of free -SH groups of one or both free cysteines in SOD 1, we propose to develop new transgenic mouse model that over expresses a mutated SOD 1 (C6A/C1 1 1S/G93A). In this transgenic mouse model, two free cysteines in human SOD1 are replaced by an alanine and a serine. Absence of inclusions/aggregates will indicate a role for free cysteines; Absence of disease as well as inclusions/aggregates may indicate a causative role of the free cysteines (-SH groups). AIM 2: We recently made a new transgenic mouse model that over expresses a truncation mutation in SOD 1 (L126Z). These mice developed a typical ALS-like phenotype and pathology. These results provide the experimental evidence that only a part of the SOD1 polypeptide, rather than entire SOD1 protein, is sufficient to produce the neuronal toxicity and cause motor neuron degeneration. We plan to define the minimum fragment of SOD 1 essential for toxicity so that further studies into the pathogenesis of ALS may be facilitated. To achieve this goal we propose to develop additional transgenic mouse lines that over express successively smaller fragments of SOD 1 polypeptide to determine the smallest segment of SOD 1 that causes ALS. -

Principal Investigator: DESHMUKH, MOHANISH
Grant Number: 5R01NS042197-04
Title: Mechanism of Neuronal Competence To-Die-By Apoptosis

Abstract: Programmed cell death (PCD), which results in apoptosis, occurs widely during neuronal development and is also observed in pathological situations of stroke, spinal cord injury, and neurodegenerative disease. The mechanism of neuronal PCD has been extensively studied in sympathetic neurons that undergo apoptosis after nerve growth factor (NGF) removal in culture. A critical factor regulating apoptosis in many cells is the cytochrome c-dependent activation of caspases. Although necessary in sympathetic neurons, cytochrome c release is not sufficient to induce apoptosis after NGF deprivation. We have recently demonstrated that a novel, uncharacterized event, called the "development of competence," is needed, along with cytosolic cytochrome c to induce caspase activation and apoptosis in these neurons. We shall examine whether the development of competence event is also important in other models of neuronal apoptosis and test the specific hypothesis that the requirement of development of competence to induce apoptosis is a phenomenon unique to postmitotic cells. We shall also examine the signaling pathway activated after NGF deprivation that leads to the development of competence. Since our preliminary results suggest that the c-jun-N-terminal kinase (JNK) signaling pathway is important in regulating competence in sympathetic neurons, we shall focus specifically on components of this signaling pathway. Lastly, we shall examine the molecular mechanism of development of competence. Our recent data suggest that competence may be controlled by an inhibitor of apoptosis protein (IAP) like activity. We shall examine this hypothesis and test the specific importance of Smac, a recently identified inhibitor of IAPs, in regulating the development of competence in neurons. These studies will provide an understanding of the biological importance and mechanism of development of competence in promoting neuronal apoptosis. Knowledge of this pathway may also identify targets for the development of strategies to suppress apoptosis and ameliorate the consequences of neuronal injury and neurodegenerative disease. -

Principal Investigator: DICKSON, DENNIS W
Grant Number: 2P50NS040256-06
Title: Genetics and Molecular Biology of Parkinsonism

Abstract: The Udall Center for Excellence in Parkinson's Disease Research at the Mayo Clinic is an integrated, multidisciplinary center that studies the Genetics and Molecular Biology of Parkinsonism. The Center draws upon the clinical strengths of the Mayo Clinic Movement Disorder Section as well as epidemiologic and longitudinal studies of Parkinson's disease (PD), dementia with Lewy bodies and aging that provide clinical material for research projects. The Clinical Core is a multi-national effort to identify and characterize multiplex families with PD for genetic studies of PD. The Clinical Core also recruits and follows sporadic PD patients and arranges for postmortem studies. The Genetic Core provides genetic screening and performs genome wide linkage studies of familial PD. When permission is granted, samples are submitted to the NINDS DNA repository. The Neuropathology Core performs postmortem evaluations of PD, provides histologic support for projects and provides postmortem material collected through several different avenues for the research projects. Project 1 builds upon progress from the previous funding period demonstrating multiplication of the alpha-synuclein gene (SCNA) in autosomal dominant, early-onset PD and focuses on population genetics of SNCA, characterization of SNCA multiplications (including the size and genes within the multiplication regions), and measuring temporal and regional alpha-synuclein expression in normals and a-synucleinopathies. Project 2 is a clinicopathologic study that determines the frequency and clinical expression of Lewy bodies in normal individuals using the Mayo Medical Records Linkage System, with studies on the role of neuronal loss, inflammation and tau on clinical features. Project 3 uses cell lines that inducibly express alpha-synuclein as well as mitochondrial toxins, such as rotenone, to study truncated and aggregated alpha-synuclein with the goal of determining the role of interacting proteins in aggregate formation and the effects of aggregates on proteasome function and gene expression.-

Principal Investigator: DIXON, C EDWARD
Grant Number: 3R01NS033150-09S1
Title: CHRONIC CHANGES IN NEUROTRANSMISSION FOLLOWING TBI

Abstract: Unavailable

Principal Investigator: D'MELLO, SANTOSH R
Grant Number: 5R01NS040408-03
Title: Signaling Pathways Regulating Neuronal Survival

Abstract: (provided by the applicant): Apoptosis is a specific mode by which cells of all types including neurons, die. While a normal feature of the developing nervous system, apoptotic death of neurons also occurs in neurodegenerative diseases, following stroke and traumatic injury, and upon exposure to neurotoxins. In these cases, apoptosis is undesirable and often leads to serious neurological deficits. The mechanisms by which these different physiological and pathophysiological stimuli abrogate the signaling pathways that normally maintain neuronal survival are far from clear. The signal transduction pathways mediating cell survival and the molecular components that comprise them can be conveniently studied in culture. Such studies have identified many molecules that are likely to be important in regulating survival of neurons in vitro as well as in vivo and which might be affected by neurotoxic stimuli or in neuropathologic conditions. The goal of this application is to examine the role of two known survival-regulatory molecules-the Akt kinase and the nuclear factor-KB (NF- kappaB) transcription factor-in a well established paradigm of neuronal apoptosis that uses cultures of rat cerebellar granule neurons. Survival of these neurons in culture can be maintained by at least four factors-elevated extracellular potassium (high K+ or HK), IGF- 1, cyclic AMP, and lithium. Although activating distinct molecules at the cell-surface, our hypothesis is that the signaling pathways utilized by these different survival factors converge on Akt and/or NF- kappaB. The specific aims of the application are as follows: 1. Knowing that Akt is necessary for IGF-l- mediated survival, to determine whether it is also involved in survival promotion by HK, cyclic AMP, and lithium. 2. To determine the mechanism by which NF- kappaB mediates survival by HK and to examine whether it is also required for survival by IGF- 1, cyclic AMP, and lithium. Special emphasis will be placed on the roles of the transcriptional coactivator, CBP, and the NF-kappaB inhibitor, IkappaB-B. 3. To determine the relationship between Akappat and NF-kappaB activation in the inhibition of apoptosis. -

Principal Investigator: DUGAN, LAURA L
Grant Number: 5R01NS041796-04
Title: UCP5-- Balancing Metabolism and Oxidation in Aging Brain

Abstract: Age is the single greatest risk factor for most neurodegenerative disorders, even those that are genetically based. This delayed onset is believed to reflect an interaction between the risk factors for a neurodegenerative disease, and the aging process itself. Oxidative damage to mitochondrial DNA accumulates in brain of older individuals in many species, including man. This observation has led to the speculation that oxidative injury to mitochondria causes loss of mitochondrial metabolic reserve during aging, and that this contributes to the age-dependent onset of neurodegenerative processes. One class of proteins uniquely situated to contribute to, or modify, these age-dependent changes in mitochondrial function are the mitochondrial uncoupling proteins (UCPs). Mitochondrial uncoupling proteins are specifically designed to impair the efficiency of energy production by mitochondria to produce heat. Outside the nervous system, UCPs regulate body weight, temperature, and the response to starvation. Recently, however, we and others have shown that these proteins also regulate mitochondrial free radical production. Three UCPs (UCP2, 4, and 5) are expressed in brain, where their function(s) is essentially unknown. Our laboratory has been studying UCP5, and has determined that it is a neuronal protein with high expression in the forebrain of both mouse and man. We also found that over-expression of UCP5 in neurons decreased mitochondrial free radical production, a potentially beneficial effect, but decreased the efficiency of mitochondrial function and enhanced the vulnerability of neurons to injury and subsequent degeneration. We hypothesize that UCP5 in brain may be a two-edged sword which trades lower mitochondrial free radical production for greater mitochondrial metabolic inefficiency. We propose to determine whether expression and/or activity of UCP5 is altered in brain during aging. We will also determine whether this results in 1) constitutively higher levels of free radical production by mitochondria in older brain, and 2) increased vulnerability of brain to metabolic stress when UCP5 expression is induced. We will first identify factors, such as hormones or caloric restriction, which regulate expression and activity of UCP5. We will then use biochemical and fluorescence imaging techniques to evaluate mitochondrial function and free radical formation. Initial experiments will be performed in cultured neurons with modified levels of UCP5 or after treatment with agents to modify UCP5 levels or activity. We will then look at how altering UCP5 expression/activity impacts mitochondrial function and free radical production in brain of old mice. For many of these experiments, we will use Thy1-YFP mice,

Principal Investigator: DUNAH, ANTHONE W
Grant Number: 1K01NS049006-01
Title: REGULATION OF NMDA RECEPTOR TRAFFICKING BY DOPAMINE

Abstract: This grant is a request for a NINDS Career Development Award for Minority Scholars in Neuroscience (K01) to investigate the Regulation of NMDA Receptor Trafficking by Dopamine. Interactions between the dopaminergic and glutamatergic systems in the striatum have implications for the pathogenesis and treatment of Parkinson's disease. My previous work has revealed significant modifications in the properties of striatal NMDA glutamate receptors in animal models of Parkinson's disease. Intriguingly, the alterations in striatal NMDA receptors occur at the level of assembly, phosphorylation and synaptic localization of the subunit proteins, and involved redistribution of receptors between sub-cellular compartments. Furthermore, we recently reported evidence for a rapid dopamine D1 receptor dependent mechanism for the trafficking of striatal NMDA receptors from intracellular compartments to the post-synaptic membrane. The molecular mechanisms for the dopamine D1 receptor mediated sub-cellular trafficking of NMDA receptors in the striatum remain largely unknown. Therefore, I will apply my molecular neuroscience and neuropharmacology backgrounds to experimentally explore and unravel the dopamine receptor dependent molecular mechanisms and signaling pathways underlying the trafficking of striatal NMDA glutamate receptors to brain synapses in primary cell culture system. As a research fellow, I have gained knowledge and received proper training in molecular mechanisms of dopamine and glutamate mediated signal transduction pathways in both in vivo and in vitro systems. The proposed career development program will further my understanding of how the dopamine and glutamate systems in the striatum interact and lead to the pathogenesis of Parkinson's disease. This career development program along with my assembled team of scientists will continue to contribute to my professional and intellectual growth, and eventually establish myself as an independent investigator. The findings from this research proposal may ultimately lead to the development of new therapeutic options for human Parkinson's disease.-

Principal Investigator: EL-KHOURY, JOSEPH
Grant Number: 5K08NS041330-04
Title: Chemokines and microglia in Alzheimer's disease

Abstract: (Applicant's Abstract): The senile plaque is a pathological hallmark of Alzheimer's disease (AD). It is composed of beta amyloid fibrils (fAbeta), activated microglia, astrocytes and degenerating neurons. Data from patients and animal models of AD indicate that accumulation of microglia in senile plaques contributes significantly to neuronal degeneration. A key step is the migration of microglia to sites of fAbeta deposition. A likely course of events includes: (1) local microglia and astrocytes bind to fAbeta deposited in their vicinity, (2) this induces them to produce chemoattractants that recruit additional microglia, (3) recruited microglia adhere to fAbeta, become activated to produce neurotoxins, and other inflammatory mediators that cause neuronal damage. (4) Activated microglia are then retained in the senile plaques and continue to produce neurotoxins. We propose to investigate the mechanism of recruitment, activation and retention of microglia in senile plaques in AD. For this purpose we will use: (a) an in vitro model for microglial and astrocyte interactions with fAbeta, (b) post-mortem human brain specimen of patients with AD, and (c) transgenic APP mice (Tg2576) that develop AD-like pathology. We propose three specific aims: Aim 1. Identify chemoattractants produced by microglia and astrocytes interacting with fAbeta and determine their mechanism of production. We have preliminary data indicating that two chemokines, MCP-1 and fractalkine, can mediate migration of microglia to sites of fAbeta deposition in vitro. Aim 2. Study the effect of MCP-1, fractalkine and other chemoattractants; identified in aim 1 on key microglial functions important in the pathogenesis of AD. We will determine the role(s) of these chemokines in fAbeta-mediated activation of microglia to produce neurotoxins, and in microglial retention at sites of fAbeta deposition. Aim 3. Analyze the effect of targeted disruption or upregulation of MCP-1, CCR2, fractalkine or CX3CR1 on AD-like pathology in transgenic APP mice Tg2576. We will cross breed mice with targeted disruption or up-regulation of MCP-1, CCR2, and fractalkine genes with transgenic APP mice Tg2576 and test the resultant double transgenic mice for markers of AD. Chemokines and their receptors are attractive therapeutic targets in many inflammatory processes. Understanding the role of chemokines in recruitment and activation of microglia in AD may lead to exciting novel therapeutic targets to delay or stop the progression of AD by delaying or inhibiting the accumulation or activation of microglia at sites of deposition. -

Principal Investigator: ELLIOTT, JEFFREY L
Grant Number: 5R01NS040911-04
Title: Neuronal-glial interaction in the pathogenesis of ALS

Abstract: Because it is motor neurons that invariably die in amyotrophic lateral sclerosis (ALS), most attention has focused on these cells as the primary site where pathophysiologic injury is initiated. However, evidence from human autopsy studies and a transgenic mouse model of familial amyotrophic lateral sclerosis, suggests a potential role for glia in the pathogenesis of disease. To address the cell specific origin of mutant (m) Cu/Zn superoxide dismutase (SOD1) induced disease, we have generated lines of transgenic mice using glial or neuronal specific promoters which allow expression of MSOD1 restricted to either neuronal or glial population. These lines do not develop motor weakness, raising the possibility that disease expression requires both neuronal and glial dysfunction induced by mSOD1. To test this hypothesis, we will determine whether crossing glial and neuronal restricted transgenic lines expressing mSOD1 will reconstitute the disease process in mice and lead to motor neuron degeneration. We will also use a spinal cord organotypic slice model of motor neuron degeneration to address specific mechanisms underlying interactions in fALS, we will generate chimeric mice from wild type and conventional mSOD1 mice, as well as derive chimera from conventional mSOD1 mice and either glial or neuronal specific mSOD1 transgenic liens. These experiments will determine whether glial/neuronal dysfunction involves cell-cell autonomous processes and ascertain whether the disease can be rescued by normal functioning glia. Although the exact mechanism of mSOD1 toxicity is still unknown, recent evidence has supported a critical role for zinc and copper ions. Because neurons and glia both express a repertoire of genes related to zinc/copper binding including the metallothioneins (MTs), we predict that both cell types will manifest abnormal MT expression patterns. In addition, we hypothesize that targeted deletion of neuronal or glial MT genes will significantly accelerate mSOD1- induced disease. Overall, these experiments will test the hypothesis that both neuronal and astroglial dysfunction is required for manifestation of disease in a transgenic murine model of fALS. These results will provide critical insights into mechanisms underlying human motor neuron disease and have important implications for future therapeutic interventions. -

Principal Investigator: ESTEVEZ, ALVARO G.
Grant Number: 5R01NS036761-07
Title: PEROXYNITRITE AND SOD IN MOTOR NEURON APOPTOSIS

Abstract: Our long-term goal is to understand how mutations to SOD can increase oxidative stress and cause the death of motor neurons in amyotrophic lateral sclerosis (ALS). We have shown that endogenous formation of the peroxynitrite by the diffusion-limited reaction between superoxide and nitric oxide induces apoptosis in cultured embryonic rat motor neurons deprived of trophic support. Both inhibitors of nitric oxide synthesis as well as Cu, Zn superoxide dismutase (SOD) delivered intracellularly with liposomes protect motor neurons from apoptosis. These data indicate that the interaction between nitric oxide and superoxide has a role in motor neuron apoptosis. Mutations to SOD are implicated in the selective degeneration of motor neurons in ALS and expression of ALS-SOD mutants in transgenic mice produces motor neuron disease. A common phenotype among the ALS-SOD mutations so far investigated is to decrease the affinity for zinc. We have shown that zinc-deficient SOD is both less efficient at scavenging superoxide and a better catalyst of tyrosine nitration. Furthermore, the copper in zinc-deficient SOD can act as a non-specific one-electron oxidase, robbing electrons from antioxidants like ascorbate and glutathione that can be transferred to oxygen to produce superoxide. In the presence of NO, zinc-deficient SOD can catalyze the formation of peroxynitrite. In the previous cycle of funding, we have shown that zinc-deficient SOD induces apoptosis in motor neurons by a nitric oxide-dependent mechanism. For the renewal, our first aim is to further investigate the mechanisms by which zinc-deficient SODs can kill cultured motor neurons and to determine what can protect motor neurons from this toxicity. Our second aim is to characterize the source or sources of superoxide induced in motor neurons by trophic factor is to characterize the source or sources of superoxide induced in motor neurons by trophic factor withdrawal. Our third aim is to test the role of tyrosine nitration by peroxynitrite in the death of motor neurons induced by either trophic factor deprivation or by zinc-deficient SOD. Completion of the specific aims will provide a mechanistic basis for explaining how motor neurons are particularly vulnerable to SOD mutations and establish a link between sporadic and familial SODs. -

Principal Investigator: FENG, JIAN
Grant Number: 5R01NS041722-04
Title: Parkin--In vivo Function and Role in Parkinson's Disease

Abstract: Parkinson's Disease (PD) is one of the most frequent neurodegenerative disorders. It is an extrapyramidal movement disorder characterized by the progressive loss of dopamine (DA) neurons in substantia nigra (SN). Recent progress in linkage studies on patients with familial PD has led to the identification of several genes implicated in this disease. One of these genes, parkin, is linked to Autosomal Recessive-Juvenile Parkinson's Disease (AR-JP). Deletions, truncations, and point mutations of parkin in AR-JP patients are correlated with their PD symptoms. However, it is not clear whether the loss of function for this gene directly causes PD, and if so, how does it occur? We propose to answer these questions by generating the parkin knockout mice. They will be used to study whether the deletion of parkin directly leads to selective loss of nigral DA neurons, and PD-like symptoms in mice. Since parkin is widely expressed in many tissues, and yet the progressive cell death is restricted to DA neurons in SN, we hypothesize that parkin may interact with specific proteins in these neurons to sustain their survival. To test this hypothesis, we propose to identify proteins that interact with parkin by using the yeast two-hybrid system. Once these proteins are identified, we will investigate their roles, in association with parkin, in the survival of nigral DA neurons. The specific goal of this project is to understand the in vivo function of parkin and its role in the etiology of AR-JP. As AR-JP and the sporadic form of PD share many similar clinical symptoms and pathological hallmarks (e.g. death of nigral DA neurons), knowledge gained from the study of parkin may shed some light on the potentially common mechanism for PD. It is our long-term objective to use this mouse genetic model to elucidate the molecular and cellular processes that lead to the progressive and selective death of nigral dopaminergic neurons and locomotor dysfunction in PD. This animal model would also be a valuable tool for the development of more effective therapeutic strategies for PD patients. -

Principal Investigator: FRIEDLANDER, ROBERT
Grant Number: 5R01NS041635-03
Title: Mechanisms and modulation of disease progression in ALS

Abstract: The functional role of the caspase cell death family in neurodegeneration, in particular ALS, has been clearly demonstrated. We have shown that caspases-1 and -3 are regulated at the transcription level in the mutant SOD1G93A transgenic ALS mouse model. Caspases-1 and -3 are specifically activated in ventral horn neurons in this mouse model. Adding relevancy to this finding, caspase-1 and -3 activation have been demonstrated in spinal cord of humans with ALS. Caspase inhibition, either by the caspase-1 dominant negative transgene, or by administration of the broad caspase inhibitor zVAD-fmk, slows disease progression and delays mortality in mutant SOD 1G93A mice. The broad goal of this study is to expand our understanding of the molecular and cellular pathways mediating neuronal cell death. This knowledge should contribute to the rational development of improved therapeutics for ALS. With this goal in mind we wish to evaluate the cell autonomous and non-cell autonomous signals modulating disease progression in ALS. The aims of this study include: 1) Evaluate the non-cell autonomous functional interaction between caspase-1 and iNOS in ALS mice. A detrimental feedback ioop appears to play a role between caspase-1-generated mature IL-1B and iNOSgenerated NO. 2) Caspase-1 and caspase-3 are regulated at the expression and activation levels. The regulation of additional caspases will be evaluated. 3) Evaluate a potential therapeutic role for minocycline in ALS. Investigate the mechanisms of minocycline-mediated neuroprotection. 4) Since neuroprotection conferred by caspase inhibition and Bcl-2 over expression occurs by acting at different stages of the cell death pathway, we hypothesize that the combination of caspase inhibition and Bcl-2 over expression will provide greater neuroprotection than either alone. A proper knowledge of the caspase-mediated pathways will aid in designing rational pharmacotherapy. Since the mechanisms of cell death in these devastating diseases appear to be shared, furthering the understanding of the mechanisms of neurodegeneration in ALS will likely result in benefits to other neurodegenerative diseases such as Huntington's, Parkinson's, and Alzheimer's disease. -

Principal Investigator: Gainer, Harold
Grant Number: 5Z01NS002723-18
Title: Cell Biology Of Neuropeptide And Catecholamine Biosynthesis And Secretion

Abstract: Unavailable

Principal Investigator: GAN, WENBIAO
Grant Number: 5R01NS041846-04
Title: In Vivo Study of Synapses in Alzheimer's Disease Models

Abstract: The aim of this study is to better understand the role of Alzheimer's disease (AD) associated synaptic proteins in regulating synaptic structure and function in vivo. Using a newly developed membrane labeling technique to visualize neuronal structures, we will study the role of amyloid precursor protein (APP) and AP peptide in structural alterations of hippocampal synapses in transgenic mouse models of Alzheimer's disease. Preliminary studies suggest that dendrites in the vicinity of extracellular amyloid deposition show various abnormalities such as local sprouting, reduced dendritic spine density, and formation of varicosities. By using time-lapse recording and calcium imaging of fluorescently labeled dendrites in living hippocampal slices, we will examine whether and how amyloid deposition leads to morphological and functional disruption of synaptic connections. To better understand the mechanisms underlying synaptic dysfunction, serial electron microscopy will be used to examine ultrastructural changes and spatial relationships of axons, dendrites, and astrocytes in the vicinity of amyloid plaques. Lastly, as age-related synapse loss occurs gradually and long-term synaptic changes in the CNS are difficult to examine, we will study synaptic alterations in accessible submandibular ganglionic neurons in living mice expressing Yellow Fluorescent Protein (YFP) in preganglionic axons and synapses. We will be able to follow YFP-labeled synapses over an extended period of time to examine the long-term effect of age and AD associated proteins on synaptic loss in living mice. Because synapse loss plays an important role in the neuronal dysfunction associated with normal and pathological aging, a better understanding of how synapses change in Alzheimer's disease models is likely to be useful in developing new therapeutic approaches. -

Principal Investigator: GIBSON, BRADFORD W
Grant Number: 5R21NS043620-02
Title: Proteomics of Complex 1 Inhibition in GSH-Depleted Cells

Abstract: Oxidative stress appears to play an important role in degeneration of dopaminergic neurons of the substantia nigra (SN) associated with Parkinson's disease (PD). The SN of early PD patients have dramatically decreased levels of the thiol tripeptide glutathione (GSH). GSH plays multiple roles in the nervous system both as an antioxidant and a redox modulator. Recently, we generated dopaminergic cell lines in which levels of GSH can be inducibly down-regulated via doxycycline (dox) induction of antisense messages against both the heavy and light subunits of gamma glutamyl cysteine synthetase (gamma-GCS), the rate-limiting enzyme in glutathione synthesis. Down-regulation of GCS results in reduction in mitochondrial GSH levels, increased oxidative stress, and decreased mitochondrial function. Interestingly, decreases in mitochondrial activities in GSH-depleted PC12 cells appears to be due to a selective inhibition of complex I activity similar to that observed in PD. This loss in enzymatic activity appears to be a result of cysteine oxidation which is reversible by the thiol-reducing agent dithiothreitol. These results suggest that early observed GSH losses in PD may be directly responsible for the noted decreases in complex I activity and the subsequent mitochondrial dysfunction which ultimately leads to dopaminergic cell death associated with the disease. The hypothesis we will examine in this proposal is that oxidation of specific cysteines within the protein subunits of mitochondrial complex I are responsible for the selective inhibition of its activity following GSH depletion. To accomplish this goal, we will employ a series of sulfhydryl-specific probes to assess the redox states of cysteine thiol groups in complex I proteins. We will use highly sensitive mass spectrometry-based proteomics methods to identify the cysteine residue(s) that are responsible for this reversible loss of mitochondrial complex I activity. We will also examine complex I proteins for other types of oxidative damage (both reversible and irreversible) that may contribute to this loss of activity. These data should provide valuable insight into the effect of oxidative stress on mitochondrial physiology as it relates to PD, particularly the structural basis for alterations in mitochondrial function. Knowledge of the molecular details of complex I dysfunction and the identification specific subunit(s) that are involved may point us towards novel therapeutic targets for the disease and provide key data on whether thiol replacement therapy is a viable option for treatment of the disease. Once identified, presence of these alterations will be assessed in future years in both an antiGSH transgenic mouse model of Parkinson disease as well as in Parkinsonian brains. -

Principal Investigator: GREENE, LLOYD A
Grant Number: 5R01NS033689-10
Title: Neurotrophic Factor Deprivation and Neuronal Cell Death

Abstract: Unavailable

Principal Investigator: GROSS, ROBERT E
Grant Number: 1K08NS046322-01A1
Title: Axon Guidance Molecules in Nigrostriatal Regeneration

Abstract: We are interested in developing strategies for the reconstitution of the dopaminergic (DA) nigrostriatal (NS) pathway that degenerates in Parkinson's disease, an important goal because of the inadequacy of current long-term treatments. Attempts to reconstruct this pathway through transplantation of precursor cells or neurons into the nigra of the adult fail, likely as a result of 1) the presence of inhibitory molecules and/or 2) the absence of trophic and guidance molecules in the adult CNS. Here we propose that an understanding of the molecular events that regulate the development of the nigrostriatal pathway will provide insights for strategies designed to improve NS pathway regeneration in the adult milieu. We propose - and have exciting preliminary data to support - that axon guidance molecules (AGMs), important molecules that direct the development of other projection pathways in the CNS, are expressed in the developing DA NS pathway. A series of experiments are proposed to elucidate the role played by AGMs and their receptors in the development of the NS pathway. Our specific aims are to: 1) Define those AGMs whose receptors are expressed in the developing axons of nigral DA neurons; 2) Define the expression of AGM ligands in relation to the developing NS pathway; 3) For those AGMs that are expressed in an appropriate anatomical relationship to influence NS development, and whose receptors are expressed in developing DA neurons, directly demonstrate chemotropic effects on fetal nigral DA neurons in vitro, and their importance in the development of the NS pathway with blocking studies ex vivo. The outcome of the experiments outlined in this proposal will hopefully be the refinement of means to counteract the inhibitory milieu of the adult injured nervous system, and recapitulate the attractive and repulsive factors that direct axonal outgrowth during development, thereby paving the way for novel reconstructive and regenerative strategies to ameliorate the symptoms of Parkinson's disease. The insights derived from these studies may also have applicability in other neurodegenerative diseases, brain injury and stroke. The research outlined is part of a customized five-year plan of training and career development for the Principal Investigator. The proposal includes active mentoring by experienced scientists, access to diverse resources, and an environment uniquely suited to help the PI develop as an independent neurosurgeon-neuroscientist. -

Principal Investigator: GUREVICH, EUGENIA V
Grant Number: 5R01NS045117-02
Title: Dopamine Receptor Trafficking in Parkinson's Disease

Abstract: Arrestins (ARR) and G protein-coupled receptor kinases (GRK) participate in homologous desensitization of many G protein-coupled receptors including dopamine receptors. The rate and extent of desensitization is sensitive to the concentration and activity of ARRs and GRKs in the cells. In their turn, the amount and activity of ARRs and GRKs can be modulated by receptor stimulation. Loss of dopamine in Parkinson's disease (PD) causes motor deficits likely related to changes in responsiveness of striatal dopamine receptors. Dopamine replacement therapy with dopamine precursor L-DOPA, although successful at first, eventually leads to motor complications. Molecular mechanisms of motor disturbances in PD and of L-DOPA- induced side effects remain elusive. Adaptations in the signal transduction pathways mediated by dopamine receptors have been implicated an in neural plasticity induced by dopaminergic denervation and L-DOPA. One of the mechanisms by which loss of dopamine or L-DOPA treatment produce behavioral responses may involve modifications in the receptor desensitization machinery. We hypothesize that loss of adequate dopaminergic stimulation in PD and subsequent non-physiological stimulation during L-DOPA therapy lead to distinct alterations in desensitization and trafficking of dopamine receptors, possibly, due to changes in expression of ARRs and/or GRKs. Specifically, loss of dopamine in PD may reduce the concentration of ARRs/GRKs in striatal neurons, thereby leading to dopamine receptor supersensitivity. First specific aim designed to test this hypothesis includes determination of ARR/GRK expression in the striatum of Pd patients and age-matched controls at post-mortem. In the second aim, the ARR/GRK expression will be studied in the rat model of PD following nigrostriatal lesion and L-DOPA treatment. The third aim focuses on feasibility of a novel way to modulate behavioral and molecular consequences of the nigrostriatal lesion and L-DOPA treatment by facilitating or inhibiting receptor desensitization and trafficking. To that end, lentivirus-mediated gene transfer of GRK2 or its inhibitor into the lesioned rat striatum will be used. The data generated by these studies may open a new promising venue of investigation eventually leading to novel strategies for management of PD. Drugs targeting the receptor desensitization machinery may prove particularly useful for prevention or alleviating of L-DOPA-induced motor complications.-

Principal Investigator: Hablitz, John J.
Grant Number: 5R01NS018145-21
Title: Dopamine Modulation of Prefrontal Cortex Excitability

Abstract: The prefrontal cortex (PFC) plays an important role in governing a number of behaviors, including motivation, emotion learning and memory. The PFC receives a dopaminergic projection from the ventral tegmental area (VTA) which has been specifically implicated in cognitive and neuropsychiatric processes. Dopamine (DA) is believed to be an endogenous neuromodulator in the cerebral cortex and to be important for normal brain function. Clinical and experimental studies have also implicated DA in the pathogenesis of a number of neurological and psychiatric disorders, including epilepsy and schizophrenia. The overall goal of this research is to understand the role of DA in the modulation of activity in local neocortical circuits. The cerebral cortex, particularly the prefrontal cortex (PFC), is heavily innervated by dopaminergic afferents, suggesting this system plays a prominent role in regulating neuronal excitability. Despite the wealth of evidence supporting a role for DA in cognition, neuropsychiatric processes and neurological disorders, our knowledge of the function of DA receptors at the circuit and single cell level is incomplete. It is hypothesized that the net effort of DA will be determined by the interaction of changes in excitatory and inhibitory synaptic activity and alterations in intrinsic neuronal excitability. Specifically, it is planned: (1) to determine if DA receptors positively modulate excitatory inputs to layer II/III PFC pyramidal neurons via a mechanism involving Dl receptors, (2) to ascertain if evoked inhibitory postsynaptic currents (IPSCs) are negatively modulated by DA. Studies will determine if this is a presynaptic effect of Dl receptors mediated by activation of PKA and (3) to characterize and compare the postsynaptic effects of DA in pyramidal cells and fast spiking interneurons. The proposed experiments will provide important new information regarding the role of specific DA receptors in the regulation of local cortical circuits. These data will be important not only in understanding normal cortical functioning, but also in understanding the mechanisms underlying abnormal processes such as schizophrenia, epilepsy and Parkinson's disease, relatedto inappropriate DA signaling. -

Principal Investigator: HALVERSON, ROBYN A
Grant Number: 5F31NS043053-03
Title: Transglutaminase Cross Linking of Tau in FTDP17

Abstract: Tauopathies are a group of neurodegenerative disorders in which filamentous tau aggregates are a hallmark pathological lesion. The recent discovery of tau mutations in FTDP-17 demonstrates that tau dysfunction can directly cause neurodegeneration. However, the mechanism underlying the formation of neurofibrillary tangles (NFT) is unclear. Work from our laboratory demonstrates that transglutaminasecatalyzed cross-linking of tau is present in NFT in two tauopathies. Transglutaminase is a calciumdependant enzyme that covalently cross-links proteins rendering them insoluble, similar to the properties of NFT. It is not known what causes the increased cross-linking of tau in tauopathies. Preliminary data from our laboratory suggest that oxidative damage and elevation of intracellular calcium produce elevated transglutaminase-catalyzed cross-links in tau. We hypothesize that oxidative stress and disruption of calcium homeostasis may contribute to the transglutaminase-catalyzed cross-linking of tau and neurofibrillary pathology of tauopathies. Our first aim is to demonstrate that transglutaminase-catalyzed cross-links are present in tau from FTDP-17 patients and mice expressing VFDP-17 associated tau mutations. The second and third aim will utilize a cell culture model to assess the effects of oxidative stress and elevated intracellular calcium on the transglutaminse-catalyzed cross-linking of mutated tau. The proposed studies will help elucidate mechanism of NFT tangle formation, therefore providing potential avenues for therapeutic intervention in a broad range of tauopathies.-

Principal Investigator: HASTINGS, TERESA G
Grant Number: 5R01NS044076-03
Title: Dopamine Toxicity and Mitochondrial Dysfunction

Abstract: This study will focus on the fundamental question of whether reactive metabolites of dopamine, may be contributing to the pathogenesis of neurodegenerative disorders such as Parkinson's disease. Dopamine has been shown to be toxic to cells both in vitro and in vivo. However, the exact mechanism associated with the toxicity is not known. Because mitochondria play a critical role in mechanisms of cell death, the proposed studies are designed to increase our understanding of the interplay between reactive metabolites of dopamine and mitochondrial function, and their ability to enhance the vulnerability of dopaminergic neurons to injury. In Aim 1, we will characterize the temporal relationship between loss of mitochondrial function and cell death following exposure to dopamine. In Aim 2, using striatonigral organotypic cultures, we will examine whether dopaminergic neurons exhibit an increased vulnerability to mitochondrial inhibition and whether dopamine contributes to this effect. Finally, in Aim 3, we will determine the identity of critical proteins modified and inactivated by dopamine quinones using mass spectrometry, with a focus on mitochondrial proteins. The outcome of these studies has potential for identifying new therapeutic targets for stopping and preventing the neurodegenerative process in Parkinson's disease.-

Principal Investigator: HORTOBAGYI, TIBOR
Grant Number: 1R13NS047105-01
Title: International Symposium on Motor Control Using TMS

Abstract: This application is a single-year request of support for an international symposium, "Mechanisms of Movement and Sensation Using Transcranial Magnetic Stimulation" (TMS) as part of the XVth biennial Congress of the International Society of Electrophysiology and Kinesiology (ISEK), Boston, June 18-21, 2004. The rationale for the symposium is that in this era of specialization, research subdisciplines on the one hand and basic researchers and therapists on the other, tend to separate. This symposium is an effort to minimize this separation. The symposium's aim is to generate a novel synthesis of basic science and clinical mechanisms of motor cortex plasticity and thus facilitate the design of rehabilitation programs. Pascual-Leone, co-chair, (US), will provide a historical perspective on TMS and rTMS. Valero Cabre (US) will discuss the effects of TMS and rTMS on the basic electrophysiological and metabolic properties of cortical neurons with reference to Parkinson's disease. Hortobagyi (US) will discuss the contralateral organization of the human nervous system. Taylor (Australia) will address the mechanisms of central fatigue in polio and chronic fatigue syndrome. Sawaki (US) will present on training dependent plasticity of the motor cortex as evidence for short-term motor memory, specifically in stroke. Rothwell (UK) will address the effect of afferent input on motor cortex organization and plasticity in healthy subjects and in patients with dystonia and hand cramps. Manto (Belgium) as co-chair will moderate the discussions. The symposium will provide maximal interaction between speakers and attendees as it will take place in a plenary session format as the only ongoing session. Through student discounts, it will provide an economical opportunity for biomedical trainees to attend. The presentations will be published in IEEE Engineering in Medicine and Biology, making a substantial impact on the field by attracting the interest of neurologists, clinical neurophysiologists, basic and clinical movement and sensation neuroscientists, physical therapists, biomechanists, biomedical engineering researchers, roboticists, educators and students from the US and abroad.-

Principal Investigator: HORVATH, TAMAS L
Grant Number: 5R01NS041725-03
Title: Uncoupling Protein 2 Promotes Neuronal Survival

Abstract: We have identified the existence of mitochondrial uncoupling protein 2 (UCP2) in homeostatic circuits of healthy rodents and non-human primates. We also showed that ectopic expression of this uncoupling protein is induced in different models of neurodegeneration, including models of Parkinson's disease, hypoxia, epilepsy or trauma-induced brain injury. The expression of UCP2 in these experiments was associated with subpopulations of neurons and microglial cells at the site of the degenerative processes and predicted cells with the longest survival after the initial insult. In our preliminary studies, UCP2 overexpressing animals had diminished levels of free radical production in the brain and responded to transection of the entorhinal pathway with suppressed caspase 3 activation. We hypothesize that the induction of UCP2 in neurons and glial cells during pathological neurodegeneration is an attempt to protect and rescue injured neurons. Three Specific Aims are proposed to test this hypothesis: Specific Aim 1 To determine the role of the UCP2 gene product in intracellular calcium homeostasis and protection of cells in vitro by studying PC12 cells and primary cultures of retinal ganglion cells with and without UCP2 transfection and primary cultures of retinal ganglion cells taken from UCP2 transgenic, UCP2 knockout and wild type mice. The effects of oxygen and glucose deprivation and glutamate agonists will be assessed on cell death patterns and intracellular calcium metabolism in these cultures. Specific Aim 2 To determine the pattern of neurodegeneration, mitochondrial uncoupling activity, cytokine and ATP production in the brains of UCP2 knockout mice, UCP2 overexpressing transgenic mice and wild type mice undergoing hypoxia-, seizure- or 1-methyl-4-phenyl- 1,2,5,6 tetrahydropyridine (MPTP)-induced neurodegeneration. Specific Aim 3 To assess the effects on phenotype development of superoxide dismutase 2 knockout animals that are crossbred with either UCP2 knockout or UCP2 overexpressing mice. In these experiments, we will follow the phenotypic alterations by assessing neuronal loss, level of mitochondrial uncoupling activity, cytokine, free radical and ATP production and intracellular calcium levels using morphological, biochemical and molecular biological approaches. The results of the proposed studies will shed light on a novel mitochondrial mechanism that plays critical roles in the suppression of neurodegeneration regardless of the initial cause of disease. This will furnish one common target for the development of drugs against a variety of neurodegenerative pathologies, including those associated with hypoxia, epilepsy, Parkinson's, Alzheimer's and Huntington's Disease. -

Principal Investigator: HSU, SHU C
Grant Number: 5R01NS038892-05
Title: Molecular Mechanisms of Neurite Outgrowth

Abstract: The precise yet dynamic networking among nerve cells is the cellular basis of many if not all brain functions. Alteration or disruption in this network is likely to result in mental deficiencies and/or illnesses. To establish and maintain this neuronal network, neurons adopt a highly specialized and flexible morphology; the formation and modulation of this specialization requires precisely targeted protein/membrane addition to designated plasma membrane domains. A molecule implicated in this targeting process is the Exocyst complex, a macromolecule essential for protein/membrane targeting and critical for neuronal development. Mouse embryos with an Exocyst subunit deletion die upon gastrulation at the onset of neural induction. As a first step to understand the molecular mechanisms of the Exocyst function in neuronal development, we identified the molecular associations of the Exocyst complex and studied its function in neuronal differentiation. We found that the Exocyst complex associates with microtubules and septins, a familty of GTPases whose members have been found to be present in Alzheimer neurofibrillary tangles and act as a substrate for Parkin, a protein implicated in the Parkinson's disease. Septins, in turn, were found to associate with the actin network. Both the Exocyst complex and the septin filament are dynamic molecules which change their subcellular localization upon neuronal differentiation in response to the MAP kinase pathway. In addition, we have also found that the Exocyst complex co-immunoprecipitates with the CDK5 kinase activator p35. We hypothesize that the Exocyst complex coordinates with cytoskeletons, under regulation by signaling molecules such as RalA and the p35/CDK5 kinase system to mediate protein/membrane targeting to designated plasma membrane domains for the generation of neuronal polarity. In this proposal, our objectives are to characterize the Exocyst complex association/coordination with cytoskeletons, to analyze how these interactions contribute to neuronal development and to study the regulation of the Exocyst complex molecular associations and function during neuronal development. These studies aim to further our understanding of the molecular mechanisms and regulations of the Exocyst complex function during neuronal development, and to guide future experimental designs to study the involvement of the Exocyst complex and its associations in neuronal regeneration and degeneration. -

Principal Investigator: HUTSON, CHE B
Grant Number: 1F31NS051163-01
Title: The Role of Inflammation in Parkinson's Disease

Abstract: Parkinson's disease (PD) is a neurological disorder characterized by the degeneration of nigrostriatal dopaminergic neurons. The cause of this degeneration has yet to be fully understood. However, there is increasing evidence that PD is the result of a complex set of interactions encompassing genetic predisposition, the innate oxidative characteristics of the nigrostriatal dopaminergic pathway and inflammation. Less than 10% of PD cases are hereditary. A subset of which has been linked to two mutations in the alpha-synuclein gene. Our laboratory has obtained a mouse that over-expresses human alpha-synuclein under the control of the platelet-derived growth factor promoter. Using this mouse as a genetic model of PD, I plan to examine the inflammatory mechanisms leading to the loss of nigrostriatal dopaminergic neurons after exposure to the inflammagen lipopolysaccharide (LPS). I hypothesize that in the context of increased alpha-synuclein expression, inflammation is detrimental to dopaminergic neurons. Furthermore, I hypothesize that LPS mediated inflammation will result in the loss of dopaminergic cells in the substantia nigra of the alpha-synuclein over-expressing murine model of PD. -

Principal Investigator: IACOVITTI, LORRAINE M
Grant Number: 3R21NS043705-02S1
Title: Neural Stem Cells Grafts in Primate Models of Parkinsons

Abstract: Unavailable

Principal Investigator: IACOVITTI, LORRAINE M
Grant Number: 5R01NS043309-03
Title: Using Stem Cells in Animal Models of Parkinson's Disease

Abstract: One promising new therapy for Parkinson's Disease (PD) involves the replacement of degenerated nigrostriatal neurons with those derived from transplanted fetal mesencephalic tissue. Although this approach has often yielded remarkable recovery of function in rats and monkeys, results in clinical trials with PD patients have been less consistent. At issue, is the relative inability to standardize a number of critical factors in human fetal transplants, including the age, type, number and integrity of cells being grafted. Consequently, finding more reliable sources of dopaminergic (DA) tissue for transplantation has become increasingly important. One direction has been to search for a line of readily available, well-characterized continually self-renewing stem or precursor cells that possess the capacity to differentiate, ideally spontaneously and with the need for little manipulation, into DA neurons, thus providing an inexhaustible and uniform source of replacement tissue. Towards this end, our preliminary findings demonstrate that grafts of embryonic mouse neural stem cells (NSCs) of the C17.2 cell can differentiate exclusively into neurons, which in a majority of cases, can express DA traits when cells are transplanted into the brain of a Parkinsonian rat. In addition, in preliminary studies using stem cells from adult human bone marrow (MSCs), we have found that nearly 100 percent of MSCs will convert into process- bearing, beta-tubulin III+ neuronal-like cells after only 1-2 hours of incubation with specific differentiation factors. If these cells also exhibit the same capacity as NSCs to respond to appropriate DA differentiation cues in vivo, patients could provide their own source of stem cells for autologous grafts in PD. Using NSC and MSC stem cell models and a multidisciplinary approach, our specific goals for this proposal are threefold: 1) Identify the conditions that promote the stable appearance of a postmitotic differentiated DA phenotype in stem cells grown in culture; 2) Identify those factors which promote the differentiation of a DA phenotype in transplanted stem cells and 3) Determine whether the DA phenotype in transplanted stem cells is stable and long lasting, and whether, it can produce functional recovery of motor deficits in a rat model of PD. The ultimate goal of this research program is a fuller understanding of the cellular and molecular processes regulating the differentiation of DA traits in stem cells and apply that knowledge to transplantation strategies for the treatment of Parkinson's Disease.-

Principal Investigator: IACOVITTI, LORRAINE M
Grant Number: 2R01NS032519-11A1
Title: Studies of Purified Dopamine Neurons

Abstract: Historically, there has been no good way to isolate DA neurons from other cells of the midbrain. Thus, missing DA neurons have been replaced by mixed cell populations following transplantation of embryonic midbrain tissue in animal models of disease and in Parkinson's patients. Although, in many cases, these transplants have provided long-term benefit, the presence of unwanted cells, such as glia, non-DAergic neurons, or even excessive numbers of DA neurons, has produced serious side effects, and in rare cases, even death. Discovering ways in which to segregate DA neurons from other cell types poses a significant challenge, but a necessary next step. In the present proposal, our plan is to take advantage of several new advances in the laboratory; including the recent cloning of 11kb human tyrosine hydroxylase gene promoter (hTH). This sequence accurately targets the expression of the reporter, green fluorescent protein (GFP) to DA neurons of the mammalian CNS. Because GFP can be directly visualized in live fetal DA neurons, this approach allows enrichment via flourescent activated cell sorting (FACS) for study in vivo and in vitro. Moreover, it is possible to adapt these purification methods to mouse stem and human progenitor cells using a lentiviral vector to transduce cells with the hTH-GFP transgene. Following their DA differentiation and FACS sorting, our goal is to study purified populations of engineered stem/progenitor-derived DA neurons in culture or after transplantation into the Parkinsonian rat. These models offer us a unique opportunity to determine the ideal number of DA neurons needed as well as the optimal conditions which contribute to their survival and growth following transplantation. Graft function will be assessed in live animals via behavioral testing and in vivo microdialysis which will be correlated with biochemical and anatomical (at the light and electron microscopic levels) changes following sacrifice. This work will hopefully lay the foundation for the development of therapeutic treatments for Parkinson's and other diseases involving compromised DA systems.
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Principal Investigator: ISACSON, OLE
Grant Number: 3P50NS039793-05S1
Title: NOVEL THERAPEUTIC APPROACHES FOR PARKINSON'S DISEASE

Abstract: Unavailable

Principal Investigator: JAKOWEC, MICHAEL W
Grant Number: 5R01NS044327-03
Title: Glutamate-dopamine plasticity in nigrostriatal injury

Abstract: The MPTP-lesioned mouse serves as an excellent model to study the mechanisms involved in the return of striatal dopamine after basal ganglia injury. The administration of MPTP to C57BL/6 mice leads to the destruction of nigrostriatal dopaminergic neurons and subsequent depletion of striatal dopamine. An advantage of MPTP-lesioning is that the degree of neuronal cell death can be titrated such that remaining dopaminergic neurons may act as a template for repair and recovery in response to the injury. Our hypothesis is that glutamate, acting through altered expression of the AMPA-subtype of receptor, activates the transcription factor phospho-CREB and leads to increased tyrosine hydroxylase expression and axonal sprouting in surviving nigrostriatal dopaminergic neurons. This research proposal is designed to define changes that take place after MPTP injury in the expression of AMPA receptors (including their phosphorylated state), the transcription factor CREB, dopamine receptors (Dl, D2, and D3), and the growth-associated protein GAP-43. The effect of blocking glutamate neurotransmission with the AMPA receptor antagonist GYKI-52466 on these parameters will be determined. The molecular tools of immunocytochemistry, western immunoblotting, in situ hybridization, and anterograde labeling will be used to define the mechanisms involved in the return of striatal dopamine. The long-term goal of these studies is to elucidate features of plasticity following injury to the brain and to identify new therapeutic interventions for the treatment of neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, and aging. -

Principal Investigator: JOHNSON, GAIL V. W.
Grant Number: 3R01NS041744-02S1
Title: Mutant huntingtin compromises mitochondrial function

Abstract: Unavailable

Principal Investigator: JOHNSON, GAIL V. W.
Grant Number: 5R01NS041744-03
Title: Mutant huntingtin compromises mitochondrial function

Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder that is caused by a pathological expansion of CAG repeats within the gene encoding for a 350 kD protein called huntingtin. This polyglutamine expansion within huntingtin is fundamental to the pathogenesis of HD, however the mechanisms by which this mutation causes the disease are unknown. One of the leading hypotheses of the etiology of HD is that mutant huntingtin directly or indirectly compromises mitochondrial function resulting in impairment of energy metabolism, increased oxidative damage and eventually neuronal death. Indeed, a marked reduction in the activity of mitochondrial complexes II and Ill, and to a lesser extent complex IV, has been detected in the striatum of subjects with HD. Further, an N-terminal fragment of mutant huntingtin has been localized to the nucleus, and there is data to suggest that mutant huntingtin can alter gene expression. Considering these and other findings, it is of fundamental importance to determine how mutant huntingtin affects mitochondrial function, and further how these changes modulate the cellular toxicity of mutant huntingtin. Our overall working hypothesis is that mutant huntingtin compromises the function of mitochondria which results in altered cellular functions and an increased sensitivity of the neurons to specific stressors. The specific aims of this proposal are to: (1) test the hypothesis that mutant huntingtin impairs mitochondrial function which sensitizes the cells to specific stressors, (2) test the hypothesis that expression of mutant huntingtin results in selective alterations in the expression of mitochondrial proteins involved in energy metabolism and (3) test the hypothesis that impaired mitochondrial function compromises proteasome activity and this results in alterations in huntingtin processing. Further, we hypothesize that this process is exacerbated in cells expressing mutant huntingtin. For these studies we will establish immortalized striatal neurons that inducibly express wild type and mutant huntingtin constructs. These studies will provide critically important data on the effects of mutant huntingtin on mitochondrial function and energy metabolism and provide insight into the mechanisms by which mutant huntingtin impairs mitochondrial function and contributes to the neurodegenerative processes in HD.-

Principal Investigator: JOHNSON, RODNEY L
Grant Number: 5R01NS020036-18
Title: Pro-Leu-Gly-NH2 and Dopamine Receptor Modulation

Abstract: Unavailable

Principal Investigator: JOHNSON, RODNEY L
Grant Number: 3R01NS020036-18S1
Title: Pro-Leu-Gly-NH2 and Dopamine Receptor Modulation

Abstract: Unavailable

Principal Investigator: JOPE, RICHARD S
Grant Number: 5R01NS037768-06
Title: Neuronal signaling : Oxidants & Alzheimers disease

Abstract: Oxidative stress may be the single most prevalent cause of neuronal dysfunction in neurodegenerative disorders, and its prevalence underscores the need to clarify mechanisms causing and attenuating the deleterious effects, the overall goals of this project. We report exciting and novel results: (1) DNA damaging agents that elevate p53 cause a novel mechanism of activation of the pro-apoptotic glycogen synthase kinase-3b (GSK3b). (2) Oxidative stress induces RGS2 (Regulator of G-protein Signaling 2) expression, a G-protein GTPase-activating protein, providing a mechanistic basis for impaired signaling. (3) Stimulation of muscarinic receptors greatly attenuates oxidative stress-induced apoptosis, remarkably as effectively as a general caspase inhibitor. These results provide important new insights about mechanisms that contribute to oxidative stress-induced impairments and about mechanisms capable of attenuating the deleterious effects. Specific Aim 1 will test the hypothesis that oxidative stress and DNA damage activate p53-mediated signaling encompassing recruitment of GSK3b by a novel activation mechanism. We will test the hypotheses that p53-induced activation of GSK3b leads to inhibition of survival-promoting transcription factor substrates of GSK3b, and promotes responses to p53, identify the p53-binding domain on GSK3b, determine if p53 binding alters the association of GSK3b with other proteins, identify the GSK3b-binding domain on p53 and determine if GSK3b binding alters p53 functions. Specific Aim 2 will test the hypothesis that oxidative stress and DNA damage induce the expression of RGS2 which attenuates muscarinic receptor-coupled signaling and facilitates oxidative stress-induced apoptosis. We will identify the signal mediating H202-induced increases in RGS2, Determine if H202-induced increases in RGS2 impair muscarinic receptor-coupled signaling, and test if IGS2 expression is pro-apoptotic role after oxidative stress. Specific Aim 3 will test the hypothesis that stimulated muscarinic receptors protect cells from oxidative stress, identify the blocked site in -1202-inducedsignaling, test the hypothesis that muscarinic receptors provide protection from other apoptotic conditions, identify the signaling pathways activated by muscarinic receptors providing protection, and test the hypothesis that activation of Rho family small G-proteins is protective. -

Principal Investigator: KALYANARAMAN,
Grant Number: 2R01NS039958-05
Title: Role of Neuronal NOS & Superoxide in Neurodegeneration

Abstract: Long-term goal: The broad objectives of this renewal are to understand the mechanism(s) by which mitochondrial neurotoxins such as 1-methyl-4-phenylpyridinium (MPP+) selectively destroy dopaminergic neurons in the substantia nigra, leading to the development of Parkinson's disease (PD). Reactive oxygen and nitrogen species (ROS/RNS)-mediated damage has been implicated in age-related neurodegenerative diseases like PD. Hypothesis: (i) MPP+ generates mitochondria superoxide (02*) and hydrogen peroxide (H202), and inactivates mitochondrial iron-sulfur-proteins (e.g., aconitase). This stimulates transferrin receptor (TfR)-mediated uptake of iron. (ii) MPP+-induced H202 and iron transported through TfR cause enhanced degradation of tetrahydrobiopterin (BH4), an essential co-factor for neuronal nitric oxide synthase (nNOS), tyrosine hydroxylase (TH), and dihydropteridine reductase (DHPR) activities. BH, depletion causes "uncoupling" of nNOS to form O2* and inactivation of TH and DHPR leading to dopamine depletion. (iii) MPP+-induced O2*, H2O2, and Tf-iron stimulate aggregation of a-synuclein, a neuronal presynaptic protein leading to apoptosis or programmed cell death. Aims: 1.) Investigate the effect of TfR-dependent iron and mitochondrial ROS in neuronal cell apoptosis in response to MPP+. 2.) Assess the modulatory effect of BH4 depletion on nNOS-generated nitric oxide ('NO)/O2* ratio and on BH4-dependent enzyme controlling dopamine synthesis. 3.) Elucidate the role of ROS, Tf-iron and BH4 depletion on a-synuclein aggregation and apoptosis in neuronal cells treated with MPP+. Methods: We will use both dopaminergic and non-dopaminergic cells (neuroblastoma and cerebellar granule neurons). The following redox-parameters will be measured: GSH and lipid peroxides; aconitase, complex-I, and iron-regulatory activities; TfR expression and 55Fe uptake; a-synuclein expression and aggregation; caspase activation and apoptosis. ROS/RNS will be determined by fluorescence and spin-trapping techniques. Significance: PD affects about 1% of population over the age of 50. Emerging data allude to environmental mitochondrial toxins as a causative factor. Novelty: This proposal sheds new light on the synergistic role for MPP*-induced mitochondrial ROS, iron, BH4-induced nNOS uncoupling, dopamine depletion and alpha-synuclein aggregation in neuronal toxicity of PD and other mitochondrial diseases.-

Principal Investigator: KANG, UN Jung
Grant Number: 5R01NS043286-02
Title: The neuroprotective effect of tetrahydrobiopterin

Abstract: While multiple etiologies are likely to account for Parkinson's disease (PD), the core pathogenic feature is degeneration of dopaminergic neurons, particularly those in the substantia nigra pars compacta (SNpc), with shared common final pathways involving oxidative damage, mitochondrial dysfunction, or both. Therefore, one may hypothesize that dopaminergic neurons in the SNpc are selectively vulnerable to oxidative stresses and/or mitochondrial disruption and understanding the mechanism of this selectivity may reveal the pathogenesis. However, our data show that ventral mesencephalic dopaminergic neurons in culture have an enhanced antioxidant capacity, as they are better able to resist oxidative stresses such as glutathione depletion and peroxide treatment than nondopaminergic neurons. In addition, their enhanced antioxidant capacity is reflected in lower reactive oxygen species (ROS) and higher reduced glutathione levels than nondopaminergic neurons. We hypothesize that an enhanced antioxidant capacity is essential for the survival of dopaminergic neurons that may be subjected to increased oxidative stress exerted by dopamine and its metabolites. We postulate that disruption of this innate antioxidant capacity makes them vulnerable to additional environmental insults and thereby leads to selective degeneration. We noted that the enhanced antioxidant capacity in ventral mesencephalic dopaminergic neurons is due to tetrahydrobiopterin (BH4), which is the cofactor for tyrosine hydroxylase, the enzyme producing dopamine, but also lowers superoxide levels, partly be direct scavenging effect and modulates mitochondrial function. First, We will study the effect of BH4 on mitochondrial bioenergetics and function including initiation of death pathways. Second, we will examine the role of BH4 on NO and superoxide generation and in modulating other endogenous antioxidant systems. Third, the neuroprotective function of BH4 against PD models such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, rotenone toxicity, and glutathione depletion will be tested in vivo and in organotypic slice cultures, using hph-1 mice that are deficient in BH4, production.-

Principal Investigator: Kanthasamy, Anumantha
Grant Number: 5R01NS045133-02
Title: CASPASES, MITOCHONDRIAL FUNCTION AND PARKINSON'S DISEASE

Abstract: Parkinson's disease (PD) is a major neurodegenerative disorder affecting approximately 2% of the population over age 50, and the number of annual PD cases continues to rise along with the median age of the population. As the population in our society ages, we face the regrettable reality that effective medical treatment strategies for major chronic neurodegenerative disorders, including Parkinson's disease, are lacking. Determining the mechanisms of etiopathogenesis and selective nigrostriatal degeneration in PD is a formidable challenge. Emerging epidemiological and case control studies suggest that environmental factors, especially pesticides, are dominant risk factors in the etiology of sporadic, geriatric-onset Parkinson's disease. In this proposal, our preliminary data reveal that dopaminergic cells are susceptible to Dieldrin (a potential environmental risk factor for development of PD) -induced apoptosis, in which oxidative stress plays a causal role. We have also uncovered a novel apoptotic pathway involving caspase-3 dependent proteolytic cleavage of protein kinase Cdelta (PKCdelta) that not only mediates apoptosis in dopaminergic cells, but also influences key cellular events such as amplification of the apoptotic cascade through positive feedback activation and hyperphosphorylation of alpha-synuclein. We will extend our preliminary findings by pursuing the following Specific Aims: (I) characterize mitochondrial dysfunction and the subsequent activation sequence of key proapoptotic factors during dieldrin-induced oxidative stimulation in the mesencephalic dopaminergic cell model of Parkinson's disease, (ii) establish the proapoptotic function of caspase-3 dependent proteolytic activation of PKC5 in Dieldrin induced dopaminergic degeneration and to further investigate mechanisms underlying positive feedback amplification of the apoptotic signaling cascade by PKCdelta, (iii) obtain evidence to support the hypothesis that proteolytically activated PKCdelta hyperphosphorylates alpha-synuclein and thereby promotes protein aggregation, (iv) examine whether chronic exposure to Dieldrin in animal models induces caspase-3 dependent proteolytic cleavage of PKCdelta, alpha-synuclein aggregation, Lewy body formation and apoptotic cell death of dopaminergic neurons in the substantia nigra, and finally, (v) confirm the involvement of PKCdelta in nigral dopaminergic degeneration by using PKCdelta knockout animals and by targeted over-expression of PKCdelta and alpha-synuclein using a lentiviral delivery system in animal models. Together, results from the proposed systematic investigation will demonstrate the involvement of mitochondrial dysfunction, oxidative stress, apoptosis and

Principal Investigator: KAPLITT, MICHAEL G
Grant Number: 1K08NS044978-01A2
Title: PTEN Anti-Oncogene: Neuronal Function and Toxicity

Abstract: The PTEN anti-oncogene is among the most frequently mutated genes in malignant brain tumors. Normally, PTEN is a lipid phosphatase which blocks malignant phenotypes primarily by inhibiting the PI3 Kinase/AKT pathways, but PTEN can also act as a protein phosphatase. PTEN is expressed in brain late in development, and neuronal expression continues throughout adult life. Although loss of PTEN can cause neuronal hyperplasia, little is known about the role of PTEN in neuronal development or in normal neurons. Pathways influenced by PTEN suggest that this anti-oncogene may increase neuronal sensitivity to toxicity and/or degenerative processes, which is supported by our preliminary data. This proposal will first determine whether PTEN can modulate sensitivity of cultured neuron-like cells to toxins used in models of Alzheimer's disease and Parkinson's disease. While studying this hypothesis, we have unexpectedly found that PTEN blocks NGF signaling in PC12 cells, and this appears to be at least partially due to inhibition of expression of trkA and p75 NGF receptors at the protein and mRNA levels. DNA microarray then revealed that PTEN can inhibit expression of several genes, including tyrosine hydroxylase and GTP cyclohydrolase 1. Since this may also have implications for neuronal function and for Parkinson's disease, the second Aim of this proposal will also explore the mechanism by which PTEN inhibits expression of these genes. The final Aim of this proposal will explore the effect of age and neurotoxins used in models of neurodegenerative disorders on PTEN levels and function to determine the biological relevance of data generated from the first two Aims. These studies and my development as an independent clinical scientist will be significantly advanced by Dr. M. Flint Beal, who will serve as my sponsor and who is a leading expert in neuronal degeneration in PD and AD. Additional mentoring by Dr. Eric Holland, a leading expert on anti-oncogene signal transduction, will also add significantly to my scientific growth and will also help me to realize many of the Specific Aims of this proposal. The environment at Cornell and the strong support of my institution will permit me to focus upon these studies with minimal distractions. My scientific background is substantial, and this will facilitate realization of the goal of this project. This plan outlined in this award will, however, enhance previously underserved aspects of my education while focusing on an important scientific question, in order to promote a successful transition to scientific independence.-

Principal Investigator: KENNEDY, MARY B
Grant Number: 5R01NS028710-15
Title: Molecular Structure of CNS Postsynaptic Densities

Abstract: Derangements in synaptic transmission are an important part of the pathology of several neurological mental diseases including epilepsy, schizophrenia, depression, and perhaps Alzheimer's disease. Much of delicate regulation of synaptic strength that is important for information processing and storage occurs through biochemical regulation of the postsynaptic membrane. The signaling protein complexes that carry out this regulation are associated with a postsynaptic structure called the postsynaptic density (PSD), a large, fibrous specialization of the submembrane cytoskeleton that adheres to the postsynaptic membrane opposite presynaptic terminals. Previous work on this application has focused on the identification of proteins that make the PSD. Here we propose to extend our studies by determining the regulatory roles of two prominent proteins that we discovered in the PSD, whose functions are still uncertain. One of the proteins, synGAP, is a ras GTPase activating protein that accelerates the inactivation of ras. We have found that synGAP is tightly associated with the NMDA-receptor signaling complex, and is inactivated by phosphorylation by CaMKII CaMKII is, in turn, activated by the calcium ion that flows through active NMDA receptors. We postulate based on our preliminary data, that inactivation of synGAP triggered by activation of NMDA receptors potentiate the actions of neurotrophins such as BDNF at glutamatergic synapses. We will carry experiments to test this hypothesis, comparing neuronal cultures from mutant mice that are missing synGAP protein to cultures from wild type littermates. We will also investigate the effect of the synGAP deletion on assembly of the NMDA receptor-associated signaling complex during synaptic development. The second PSD protein that we propose to study, densin-180, is a member of a family of proteins that contribute to the formation of polarized membrane domains. We have found that densin forms a ternary complex with the actin-associated protein alpha-actinin and CaMKII. We will test the hypothesis that densin, in addition to NR2 subunits of the NMDA receptor, is an anchoring site for CaMKII in the PSD. We will also determine whether densin contributes to the translocation of CAMKII to the PSD that occurs upon stimulation of hippocampal neurons with glutamate. -

Principal Investigator: KINDY, MARK S
Grant Number: 5R01NS039588-05
Title: Oxidized Lipoproteins in Neurodegeneration

Abstract: This proposal tests the hypothesis that oxidized lipoproteins induce neurodegeneration directly by acting on neurons and indirectly by activating microglia through a mechanism involving scavenger receptors. Oxidative stress mediated neuronal cell loss has been demonstrated in neurodegenerative disorders including Alzheimer's disease (AD) and stroke. Reactive oxygen species (ROS) can increase the rapid oxidation of lipids and proteins generating lipid peroxidation and oxidized protein products. Once formed, these oxidatively modified lipids and proteins may be the primary means by which ROS toxicity is elicited. High-density lipoproteins (HDLs) in the central nervous system are vulnerable to oxidative modification by trace metals, ROS, and enzymatic pathways. Preliminary data demonstrate the detrimental effects of oxidized HDL (oxHDL) on neuronal cells and the activation of microglial response in vitro. The specific aims of this proposal are: 1) To test the hypothesis that HDL induces neurodegeneration both in vitro and in vivo by activating ROS. We will characterize the neuronal and microglial response to oxHDL by activating oxidative stress, calcium and apoptotic pathways. 2) To test the hypothesis that oxHDL functions through interaction with scavenger receptors on neuronal and microglial cells. We will examine cell lines expressing scavenger receptors (SR) and cells isolated from SRgene-inactivation mice for altered response to oxHDL. 3) To test the hypothesis that the apolipoprotein E (apoE) genotype may affect the level of oxidation and the neuronal and microglial response to oxHDL. We will isolate apoE-specific HDL particles and determine their susceptibility to oxidation and their effects on neurodegeneration. 4) To test the hypothesis that oxidized HDL and scavenger receptors are present in AD brain in a regional pattern related to selective vulnerability. We will examine HDL isolated from control and AD brain for oxidative status and the relationship to apoE genotype. We will also examine the expression of SR and other molecules potentially relevant to the effects of oxHDL in the AD brain. These studies should provide insights into the normal function of HDL and SR in the CNS and in the pathogenesis of AD. -

Principal Investigator: KOCHANEK, PATRICK M
Grant Number: 5R01NS038087-06
Title: Adenosine and Traumatic Brain Injury

Abstract: In traumatic brain injury (TBI), adenosine activates high affinity A1 receptors conferring anti-excitotoxic effects. After TBI, however, adenosine levels are high-activating lower affinity A2a receptors that may down-regulate A1 and confer direct neurotoxicity. In models of Parkinson's disease, A2a receptor antagonists are neuroprotective. We reported neuroprotective effects of adenosine after TBI-via anti-excitotoxic effects at the A1 receptor. However, activation of lower affinity A2a receptors could negate this benefit. Out pilot studies show that A2a receptor ko mice are neuroprotected vs wt after experimental TBI and administration of the A2a agonist CGS21680 worsens outcome. However, A2a receptor agonists increase cerebral blood flow (CBF), a finding that must be reconciled. Our clinical studies show that increases in adenosine in cerebrospinal fluid (CSF) are associated with poor outcome. A therapeutic opportunity for A2a receptor antagonists is suggested; however, this pathway must be first studied in experimental TBI. A2a receptor signal transduction is coupled to adenyl cyclase (AC). We reported progressive increases in cAMP levels in CSF after clinical TBI. Hypothesis: Treatment with A2a receptor antagonists or inactivation of the A2a receptor will improve outcome after experimental TBI. Using the controlled cortical impact (CCI) model of TBI in mice and rats, we will address five aims: (1) Determine A2a receptor dynamics after CCI in mice and rats, (2) Assess the role of the A2a receptor in determining biochemical (glutamate, ACh, cAMP), functional, and histological outcome after CCI in mice and rats, including A2a receptor ko mice, (3) Assess the effects of A2a receptor activation on CBF and cerebral metabolic rate after CCI in rats. (4) Define the role of A2a receptor-mediated activation of AC after CCI in mice and rats, (5) Determine the role of the A1 receptor in the detrimental effects of A2a agonists in CCI using A1 receptor ko mice, and (6) To bridge bench and bedside after severe TBI in humans, using CSF samples from 161 patients, we will quantify levels of the non-selective adenosine receptor antagonist caffeine (and metabolites) to test the hypothesis that acute caffeine consumption is associated with favorable outcome and reduced cAMP. These studies explore the most promising adenosine-based therapy for TBI-A2a receptor antagonists. Our bench to bedside track record ensures translation to the clinic. -

Principal Investigator: KONTOPOULOS, EIRENE
Grant Number: 1F31NS049869-01
Title: Mechanisms of Neurotoxicity in Parkinson's Disease

Abstract: Our long-term objective is to elucidate the underlying mechanisms of neuronal death in Parkinson's disease (PD). The identification of several genes exhibiting linkage to PD has not yet led to the understanding of how their protein products bring about cell death. Though in vitro studies have been instrumental in identifying potential mechanisms of neurodegeneration, their findings need to be corroborated in vivo. I propose to utilize Drosophila genetics to investigate putative protein interactions among three PD-linked genes: synphilin-1, alpha-synuclein, and parkin. My primary strategy will be to investigate the inherent toxicity of synphilin-1 and its PD-associated mutation, R621C. Furthermore, genetic interactions between both forms of synphilin and either alpha-synuclein or parkin will be examined. These efforts will culminate in the investigation of genetic interactions among synphilin-1, alpha-synuclein, and parkin. -

Principal Investigator: KOPIN, ALAN S
Grant Number: 5R21NS043692-02
Title: Evaluation dopamine receptors in Parkinson's

Abstract: Parkinson's disease (PD) results from the degeneration of nigrostriatal dopaminergic neurons. This process ultimately leads to a progressive decrease in dopamine mediated striatal signaling which manifests as a clinical syndrome characterized by bradykinesia, rigidity, tremor, and gait abnormalities. Traditional therapy for PD has aimed at restoring dopamine levels in the striatum through administration of the dopamine precursor, L-dopa. With advanced disease, L-dopa leads to dyskinesias and periods of marked fluctuation in motor activity ('on-off effect'). Alleviation of these side effects has been a major challenge and has prompted a search for alternative strategies which can provide a more stable level of dopaminergic signaling. A previously unexplored option to restore striatal dopaminergic activity and at the same time to potentially avoid the consequences of long term L-dopa administration, is through the introduction of constitutively active dopamine receptors. The laboratory of the PI has extensive experience in generating receptors with ligand independent (or constitutive) activity through the introduction of activating point mutations. These receptors have the potential to maintain dopaminergic signaling even in the absence of dopamine and/or dopaminergic agonist drugs. The premise of this application is that constitutively active dopamine receptors can be identified using in vitro assays and expressed in the striatum of rats to enhance dopaminergic signaling over an extended time interval. The objective of Specific Aim 1 is to generate and pharmacologically characterize in vitro a series of constitutively active dopamine 1 and dopamine 2 receptors. Using recombinant adeno-associated virus, the functional consequences of striatal overexpression of constitutively active dopamine receptors will be explored in rats (Specific Aim 2). Circling behavior after unilateral viral administration will be used as an index of construct activity. The methodologies utilized will include molecular (generation of constitutively active mutant receptors, expression of recombinant proteins), pharmacologic (radioligand binding, second messenger signaling assays), and behavioral approaches (assessment of circling behavior). These experiments will provide additional insight into the role of dopaminergic receptors in the striatum as well as potentially take the first steps toward the development of a new therapeutic option for Parkinson's disease. -

Principal Investigator: KOPITO, RON R
Grant Number: 5R01NS042842-03
Title: Protein aggregation and inclusion body formation

Abstract: Deposition of aggregates of misfolded protein into intracellular inclusion bodies is a prominent cytopathological feature of nearly every known neurodegenerative disease. Despite mounting genetic and biochemical evidence linking protein aggregation to pathogenesis in these and other diseases, it is unclear bow -or indeed whether- protein aggregation and inclusion body formation are primary toxic events or cytoprotective responses. My lab has recently described a general pathway by which aggregated proteins in mammalian cells are collected into specialized inclusion bodies called aggresomes (AG). The studies described in this proposal are intended to test the hypothesis that delivery of protein aggregates to AG is a specific, microtubule-dependent transport process which facilitates the neutralization and elimination of potentially toxic gene products. Towards this end, three specific aims are proposed. The first aim will use biochemical and biophysical techniques to study the cellular mechanism of AG formation to identify transport intermediates in AG formation. These intermediates will be subject to extensive biochemical, biophysical and structural characterization. The second aim of the proposed research will be to reconstitute AG formation in a cell-free system in order to identify the cytoplasmic components required for retrograde transport of protein aggregates on microtubule tracks. Finally, the last aim will investigate the role of retrograde transport in the neutralization and elimination of protein aggregates. These last studies will specifically test the hypothesis that retrograde transport of aggregated protein is linked to the lysosomal/autophagic pathway of protein degradation. -

Principal Investigator: KOTZBAUER, PAUL T
Grant Number: 1K08NS048924-01
Title: Neurodegenerative consequences of PanK2 mutations

Abstract: The candidate is an M.D./Ph.D neurologist who is currently a trainee in the Center for Neurodegenerative Disease Research. His goal is to develop additional research skills and experience needed to become an independent clinician scientist working to understand the pathogenesis of neurodegenerative diseases. The proposed research project focuses on neurodegeneration with brain iron accumulation (NBIA), which causes progressive impairment of speech, movement and cognition. At the neuropathological level, NBIA is characterized by iron accumulation, inclusion formation, signs of oxidative stress, and death of multiple neuronal populations. These features are also seen to varying degrees in other neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease. Mutations in the gene for pantothenate kinase 2 (PanK2) were recently identified in a subset of NBIA cases. The PanK2 gene encodes an enzyme involved in coenzyme A (CoA) synthesis, a critical pathway linked to a number of cellular processes, including fatty acid synthesis, energy production, and possibly, synthesis of anti-oxidant molecules. The long term objectives of this project are to understand how PanK2 mutations lead to iron accumulation, oxidative stress, inclusion formation, and neuronal death. The proteolytic processing, mitochondrial localization and in vitro catalytic properties will by characterized for mutant Pank2 proteins and compared to the wild type human PanK2 protein. Cell culture systems will be established in which PanK2 expression is eliminated and in which wild type or mutant PanK2 proteins are over-expressed. Mice that lack PanK2 expression will also be generated. Cell lines and mice lacking PanK2 expression will be examined for changes in levels of biochemical intermediates hypothesized to be dependent on PanK2 function. Finally, neuronal and non-neuronal cells lacking PanK2 will be examined for signs of increased oxidative stress, susceptibility to oxidative injury, cellular and mitochondrial import of radio labeled iron, and inclusion formation.-

Principal Investigator: KRAMER, HELMUT J
Grant Number: 5R01NS043406-03
Title: Hook proteins in membrane trafficking & neurogeneration

Abstract: Neurodegenerative diseases such as Huntington's disease, amyotrophic lateral sclerosis or Parkinson's disease share one common feature, the slow accumulation of misfolded proteins. As misfolded proteins accumulate in neurons they are not evenly distributed. Instead, they are concentrated in inclusion bodies. How these inclusion bodies are linked to the progression of neurodegenerative diseases is not well understood. One class of inclusion bodies, aggresomes, are formed at the microtubule organizing center in an active process that requires microtubule-based transport. We recently discovered that the active concentration of misfolded proteins in aggresomes involves the Hook2 protein. Hook proteins constitute a family of coiled-coil proteins which bind to microtubules and affect the organization of different organelles in mammalian cells and in Drosophila. In this grant, we will combine genetic approaches in Drosophila, cell biological approaches in mammalian tissue culture cells and biochemical experiments in-vitro to characterize shared functions of Hook proteins, as well as the specific role of Hook2 in the cellular trafficking of misfolded proteins. In Spec. Aim 1, we will determine the relevance of microtubule binding of Hook proteins using a combination of biochemical approaches in vitro and genetic experiments in Drosophila. In this context we will also explore the potential interaction of Hook proteins with the complex between cytoplasmic Dynein and Dynactin. In Spec. Aim 2, we will characterize the binding of Hook proteins to different organelles and identify the receptors that mediate these interactions. In Spec. Aim 3, we will determine the role of Hook2 in the formation of aggresomes and the potential of using dominant-negative forms of Hook2 to manipulate the aggregation of different misfolded proteins. In Spec. Aim 4, we will determine the domains of Hook proteins responsible for their polarized distribution in neurons and the role of Hook proteins in establishing neuronal polarity in rat hippocampal neurons. -

Principal Investigator: LANSBURY, PETER T
Grant Number: 1R21NS047420-01A1
Title: High Throughout Assay to Probe UCH-L1 Ligase Inhibitors

Abstract: Parkinson's disease (PD) is characterized by the presence of Lewy bodies (the cytoplasmic neuronal inclusions) and the significant loss of dopaminergic neurons in the substantia nigra, a-synuclein was identified as one major fibril component of the Lewy bodies, thus linked the accumulation of this protein to the pathogenesis of PD. Failure to regulate the concentration of a-synuclein, for example by dysfunction of the pathogenesis of PD. Failure to regulate the concentration of a-synuclein, for example by dysfunction of degradation process, can also contribute to the build-up and consequently fibrillation of the protein. A gene, PARK5, has been linked to PD are involved in proteasomal degradation pathway and it is an ubiquitin C terminal hydrolase (UCH-L 1) that hydrolyzes C-terminal ester and amides of ubiquitin and is believed to play a key role in processing polyubiquitin and/or ubiquity lated proteolytic peptide. A rare mutation (193M) of UCH L 1 that yields a 50% reduction in its hydrolytic activity has been tentatively linked to a rare early onset form of PD, at the same time a polymorphism of the enzyme (S 18Y) was indicated to reduce the risk of PD. The assumption that each enzyme expresses a single enzymatic activity in vivo, however, is challenged by the linkage of UCH-L 1 to PD. UCH-L 1, especially those variants linked to higher susceptibility to PD, causes the accumulation of a-synuclein in cultured cells, an effect that cannot be explained by its recognized hydrolase activity. UCH-L1 exhibits a second, dimerization-dependent, ubiquityl ligase activity. The polymorphic variant of UCH-L1 that is associated with decreased PD risk (S 18Y) has reduced ligase activity, but comparable hydrolase activity as the wild-type enzyme. Thus the ligase activity, as welt as the hydrolase activity of UCH-L1 may play a role in proteasomal protein degradation, a critical process for molecules ("molecular probes") that can be used to perturb UCH-L1 ligase activity in cell culture and animal models of PD. This "chemical genetic" strategy is complementary to traditional genetic approaches (e.g., knockouts and trasngenics) for understanding protein function but has a distinct advantage in that the probes are potential lead compounds for the development of novel PD therapetutics. The program detailed below will seek probes with the following activities: (1) inhibitors ofUCH-L1 dimerization, (2) inhibitors of UCH-L1 ligase activity, and (3) repressors and activators of UCH-L1 expression. -

Principal Investigator: LAU, YUEN-SUM
Grant Number: 5R01NS047920-02
Title: Impact of Exercise on Parkinson's Disease Therapy

Abstract: Parkinson's disease (PD) is a slow, progressive, debilitative, neurodegenerative disease, which has no cure. The current pharmacological therapies only temporarily mask symptoms, but do not protect neurons from further degeneration. Furthermore, chemotherapeutic agents often cause severe adverse effects and reduce the effectiveness of treatment. Numerous clinical reports have suggested that endurance exercise can slow down disease progression, and add years of independent and quality life to PD patients, or even improve the delivery and efficacy of L-DOPA treatment. Exercise therapy, or in conjunction with drug therapy at early onset of disease state, have been highly advocated by recent clinical trials. The potential health benefit and neurological mechanisms of action for exercise on PD rehabilitation have not been rigorously tested in the laboratory animal models. This research is designed to elucidate the impact of endurance exercise training on nigrostriatal dopamine (DA) neuron plasticity using a slow, progressive, and neurodegenerative mouse model of PD developed and characterized by our laboratory. This model is established based on a regimen of chronic 1-Methyl-4-phenyl - 1,2,3,6-tetrahydropyridine (MPTP) injections co-administered with probenecid, a drug that inhibits the peripheral and neuronal clearance of MPTP and potentiates the neurotoxicity of MPTP. In this model, we observed a marked decrease of nigrostriatal DA function within one week after treatment and remained low for 6 months. The animal also shows a gradual loss of substantia nigra (SN) neurons, decline of motor activity, and an accumulation of c-synuclein-immunoreactive inclusions in the SN. We further present in the application our preliminary findings supporting the feasibility and potential neuromodulatory role of endurance exercise on enhancing nigrostriatal DA transmission and PD rehabilitation using this model. In this research, we will test the following hypotheses centered on the endurance exercise, when administered at an early stage in the parkinsonian (PK) mice, will 1) improve their mobility and physical rehabilitation, 2) improve the efficacy of L-DOPA, 3) produce these effects by mechanistically causing an elevation of BDNF expression, an increase in the differentiation of DA progenitor cells, and an enhanced DA transmission and plasticity in the nigrostriatal neurons. Findings from this research should provide new insight into the development of alternative therapeutic approaches for enhancing the conventional pharmacological treatment and rehabilitation of PD. Potential benefits for using such a synergistic approach in managing PD would likely reduce the risk of drug toxicity and lower the cost of health

Principal Investigator: LAWRENCE, MATTHEW S
Grant Number: 1R43NS048786-01
Title: Genomic markers of environmental toxins for Parkinsonism

Abstract: Parkinson's disease is a prevalent and devastating neurodegenerative condition of unknown etiology. One prominent hypothesis holds that the selective loss of the nigrostriatal dopaminergic neurons characteristic of the disease results from damage from environmental neurotoxins in genetically vulnerable individuals. Identifying such environmental contributors to Parkinson s pathogenesis represents a significant public health concern. This project aims to identify the in vivo gene expression changes that occur in the primate brain in response to environmental toxins that have been implicated in the production of Parkinson's and compare these changes with the selective neurotoxin, MPTP, and with the limited knowledge of genetic abnormalities in some Parkinson's patients. Because of the unique vulnerabilities of nonhuman primates and humans to dopamine neurotoxic agents, studies in primates are essential to uncover common genetic markers of toxicity and to reveal the potential toxicity of chemicals of unknown liability. The proposed Phase I studies will test the hypotheses that transcriptional changes that accompany and precede dopamine cell death can be identified using high density gene arrays and bioinformatics in the primate nigrostriatal system in vivo following MPTP exposure. Changes in mRNA initiation of regimen of 3 doses of MPTP over 36 hours that has been established to result in Parkinsonism. Expression changes will also be assessed 6 hours after the administration of a single dose. Changes in nigrostriatal dopamine concentrations and tyrosine hydroxylase immunohistochemistry will be assessed at all time points. Additionally neurobehavioral changes will be assessed in the 20-day animals. Together these data will allow a determination of the sequence of transcriptional changes that parallel or precede histological, biochemical and behavioral events, and allow an assessment of transcriptional events related to acute versus chronic toxicity, with confirmation by quantitative RT-PCR. Defining the chronological and dose dependent gene expression changes induced by MPTP may reveal a transcriptional profile that is predictive of nigrostriatal injury from this toxin. Phase II studies will address whether similar gene expression changes and neuronal injury are seen following exposure to environmentally prevalent compounds that are postulated to be risk factors for the development of Parkinson's disease, and to integrate the resulting transcriptional data into a toxicogenornic database and potentially customized microarrays which may be applied to the assessment of compounds for their possible health risk.-

Principal Investigator: LEBLANC, ANDREA C
Grant Number: 5R01NS040965-03
Title: 17 Beta-estradiol induced caspase inhibitory factor

Abstract: The primary goal of this application is to isolate a caspase inhibitory factor induced by 17-beta-estradiol in primary cultures of human neurons. A group of mammalian cysteinyl caspases is activated in a cell-, insult- and species-specific manner during apoptosis of various cell types. In human neurons, caspase-6 is active during serum deprivation-mediated neuronal apoptosis. We have previously shown that caspase-6 activity is lethal to human neurons in culture. Now, we find that 17 -beta-estradiol but not 17-alpha-estradiol, testosterone, or epitestosterone delay caspase-6 mediated neuronal cell death (Zhang et al. 2001). 17-beta-estradiol-treated neuronal extracts directly inhibit recombinant active caspase-6 in an in vitro assay. In contrast, 17-beta-estradiol does not induce CIF nor prevent caspase-mediated cell death in astrocytes. We conclude that 17-beta-estradiol induces a caspase inhibitory factor (CIF) that is preventing neuronal apoptosis. CIF is induced through estrogen receptors via a non-genomic pathway. We show that CIP is a broad spectrum caspase inhibitor between 10 and 14 kDa in size that is fairly resistant to boiling and proteinase K in neuronal extracts. Our results indicate that 17-beta-estradiol induces a novel inhibitor of active caspases and provide an additional mechanism for the neuroprotective action of 17-beta-estradiol. In this proposal, the primary goal is to identify CIF and determine its role in neuronal survival and cell death. In aim #1, we will biochemically isolate and sequence CIF. In aim #2, we will clone CIF cDNA and obtain antibodies. We will then confirm the role of CIF in neuronal survival and against caspases (aim #3), and determine the mode of activation of CIF (aim #4) and inactivation of caspase-6 (aim #5). Finally, we will study the regulation of CIF expression in normal and AD brains (aim #6). Given the strong epidemiological link of estrogen against Alzheimer's disease and its possible prophylactic role in neuroprotection, our results suggest a novel mechanism of action of 17-Beta-estradiol that could be exploited to promote neuroprotection in injury or neurodegenerative diseases. -

Principal Investigator: Lee, Gloria
Grant Number: 5R01NS032100-08
Title: Phosphorylation and Spatial Localization of Tau Protein

Abstract: Tau is a microtubule-associated protein that is the primary component of neurofibrillary tangles in Alzheimer's disease. Several other neurodegenerative disorders also exhibit abnormal tau lesions and mutations in the tau gene cause some of these diseases. The mechanisms underlying the formation of neurofibrillary tangles are unknown, as are the mechanisms through which mutations in the tau gene cause neurodegenerative disease. Tau's primary known function has been to stabilize and promote microtubule assembly. However, neurons have several microtubule-associated proteins endowed with similar properties and yet tau is uniquely associated with neurodegenerative disease. Similarly, tau is uniquely associated with axonal development although the basis for its role in the acquisition of neuronal cell polarity is not understood. Therefore, unique properties of tau may be responsible for its role in axonal development and neurodegenerative disease. Our laboratory has approached these issues by seeking to identify new functions for tau. We have found that the amino terminus of tau is associated with plasma membrane. We have also found that in neuronal cells, tau associates with the src family non-receptor tyrosine kinase fyn and that in vitro, a PXXP motif in tau interacts with the SH3 domain of src family non-receptor tyrosine kinases. In furthering these findings, we have obtained preliminary data suggesting that tau can increase the tyrosine kinase activity of fyn and that tau associates with membrane microdomains, also known as membrane rafts. We propose to (1) determine the effect of the tau-fyn interaction on the tyrosine kinase activity of fyn and on protein tyrosine phosphorylation in neuronal cells, (2) investigate the tyrosine phosphorylation of tau during signal transduction, (3) extend the characterization of tau in membrane rafts and determine if tau's localization in membrane rafts is dependent on fyn, and (4) investigate the role of tau in membrane rafts in neuronal differentiation. We hypothesize that the interaction between tau and fyn takes place in membrane rafts and that this interaction affects fyn activity, thereby affecting signal transduction. Also, given tau's location in membrane rafts, we speculate that the tyrosine phosphorylation of tau might act to transduce extracellular signals. Signal transduction pathways involving tau and A-beta may go awry during neurodegenerative diseases, leading to inappropriate cross talk between pathways that may culminate in abnormal tau phosphorylation and polymerization. -

Principal Investigator: LEE, MICHAEL K.
Grant Number: 1R21NS049088-01
Title: Conditional Uch-L1 knockout mice

Abstract: Ubiquitination is a post-translational modification of proteins that regulate a host of important cellular processes including degradation of cellular proteins, expression of genes, and protein/membrane trafficking. Ubiquitination of proteins and the specificity of ubiquitination are mediated by three classes of ubiquitin ligases (E1, E2, and E3). In addition to the ligases, a variety of deubiquitinating enzymes (DUBs) may regulate ubiquitination in cells. DUBs include ubiquitin carboxy-terminal hydrolase L1 (Uch-L1), a protein selectively expressed in neurons and in sertoli cells of testis. Defects in Ubiquitination/proteasomal mechanisms are implicated in the pathogenesis of many neurodegenerative diseases including Alzheimer's disease and Parkinson's disease. In particular, pathogenic relationship between defects in ubiquitin/proteasomal pathway and degeneration of dopaminergic neurons are indicated by the fact that PD-associated mutations are identified in Parkin, an E3-ubiquitin ligase, and Uch-L1. In gad mutant mice, deletion of exon 7 and 8 of Uch-L1 gene leads to degeneration of neurons in the DRG and in the gracile nucleus and early lethality. However, because Uch-L1 is expressed at high levels in many neuronal populations, Uch-L1 function may be important in other neurons. Because of early lethality and general underlying movement defect in gad mice, the functional importance of Uch-L1 in a various neuronal population and as a function of aging can not be examined effectively. We propose generate a mice where the expression of Uch-L1 can be temporally and spatially regulated. Specifically, we will generate UchL1-floxed mice to conditionally silence Uch-L1 expression by mating to appropriate Cre expressing mice. As an initial test of our hypothesis, we will determine whether Uch-L1 activity is important for normal functioning and aging of the dopaminergic and noradrenergic neurons by mating. The loxP-targeted Uch1 mice will be mated to pTH-Cre transgenic mice to silence Uch-L1 expression only in the dopaminergic and noradrenergic neurons.-

Principal Investigator: LEE, TONG H
Grant Number: 5R01NS042124-02
Title: Presynaptic Mechanisms and Parkinson Treatment

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disease that follows an insidious, protracted time-course during the initiation and early progression phases (neurodegenerative cascade). Although this delayed time-course presents increased diagnostic and therapeutic challenges for the high-risk population, it also provides a window of opportunity for an early prophylactic intervention. Mechanisms underlying the progression of PD have not fully elucidated, however, recent studies have implicated, among others, a cascade involving microglia (and astrocyte) activation and subsequent increase in oxidative stress via induction of the inducible form of nitric oxide synthase (iNOS). One of the main hypotheses for this proposal is that the success of preventive intervention would critically depend not only on elucidating underlying causes (e.g., oxidative stress) but also delineating various dynamic, compensatory changes that may occur in the nigrostriatal (N-ST) dopamine (DA) neurons during the early time period. Using a rat model of progressive N-ST degeneration (partial 6-OHDA lesion in the caudate/putamen (CD/PTM)) and real-time electrophysiological and voltametric techniques, we will first characterize the early N-ST changes in neuronal activity and DA release/uptake dynamics during the first 10 days following a striatal 6-OHDA injection. Specific mechanisms to be tested include soma/dendritic and terminal DA autoreceptors, NMDA receptors basic neuronal membrane properties, and NO regulation; selective pharmacological agents and oligodeoxynucleotide antisenses will be used. Subsequently, these data will guide us in evaluating standard and experimental pharmacotherapies in preventing/reversing the electrophysiological and voltametric alterations in the N-ST DA system. It is expected that systematic delineation of the ongoing pathological process and regulatory changes in the N-ST DA neurons would facilitate formulation of treatment strategies. -

Principal Investigator: Levine, Michael S
Grant Number: 2R01NS033538-09
Title: Physiological Modulation by Dopamine in the Neostriatum

Abstract: The experiments in this proposal are designed to continue our investigations into cellular electrophysiological processes controlling dopamine (DA) modulation of responses mediated by activation of ionotropic glutamate receptors (iGluRs) in medium-sized spiny neurons of the striatum (MSSNs). The complex interactions between DA and iGluR-mediated neurotransmission within the striatum form the underpinnings of movement sequencing, motivation and reward responses, and psychological normalcy, just to provide a few examples. Imbalances in the interplay of these neurotransmitters have devastating consequences that are apparent in prevalent neurological and neuropsychiatric diseases such as Parkinson's and Huntington's diseases, attention deficit hyperactivity disorder (ADHD), schizophrenia, Tourette's syndrome, and many addictions. We have shown that DA, via D1 receptor activation enhances responses mediated by NMDA receptors while D2 receptor activation attenuates responses mediated by non-NMDA receptors (AMPA/KA). For example, when a D1 agonist was applied and a response was mediated by NMDA receptors, 98% of the time the response was enhanced. When a D2 agonist was applied and a response was mediated by non-NMDA receptors the response was attenuated 100% of the time. Other combinations (D2-DMDA, D1-non-NMDA) were less predictable. We will continue to focus on these interactions as an underlying theme, but will evaluate new areas pertaining to DA modulation. First, we will assess DA-iGluR interactions in a novel mouse model of ADHD that has the DA transporter (DAT) knocked down to 10% of basal levels. This produces a hyperDA state. Our working hypothesis is that DA modulation of iGluR transmission is altered in this genetic model and we have preliminary data to support it. Second, we will further examine mechanisms that control the predictability of DA modulation of GluR responses determining why the D2-NMDA and D1-non-NMDA receptor interactions are less predictable. Our hypothesis is that if factors controlling these interactions can be reduced, the interactions become predictable. We will use a novel mouse model in which enhanced green fluorescent protein is expressed under the control of the promoters for the D1 or D2 DA receptors or the M4 muscarinic acetylcholine receptor. This will allow electrophysiological recording in identified MSSNs that make up the direct or indirect output pathways of the striatum. Third, we will begin to dissect the NMDA receptor in MSSNs to determine how DA modulation is affected when selective subunits or their components (NR2A, NR2A-C-terminal, NR2B) have been removed or blocked pharmacologically. Our working hypothesis is

Principal Investigator: LEVINTHAL, DAVID J
Grant Number: 5F30NS043824-03
Title: The Role of MKPs in Oxidative Neuronal Cell Death

Abstract: Much of the neuronal damage resulting from ischemia is not immediate but delayed and is thought to be due, in addition to excitotoxic cell death, to overwhelming oxidative stress and subsequent apoptosis. Similarly, other debilitating diseases such as Parkinson's Disease and Alzheimer's Disease have been linked with chronic oxidative stress and neurodegeration. It is the purpose of this proposal to investigate the signaling events that occur during oxidative stress in neurons and to conceive of approaches that may prevent neuronal death in this context. Glutamate-induced oxidative toxicity, both in the mouse hippocampal cell line, HT22, and primary immature neuronal cortical neurons, is a well-studied model of oxidative stress and neuronal cell death. Little is known about the role of the mitogen-activated protein kinase (MAPK) phosphatases (MKPs) during oxidative stress in neurons. We plan to investigate changes of expression and activity of MKPs during glutamate-induced oxidative stress in both HT22 and primary cortical neurons. In addition, MKP3 and its catalytically inactive mutant, MKP3 C293S, will be transfected into both cell systems in order to investigate the ability of this phosphatase to abrogate glutamate-induced oxidative toxicity. Further experiments involving the construction of a ligand- inducible MKP3-estrogen receptor ligand-binding domain (MKP3-ER LBD) will allow the temporal control of MKP3 activity in response to tamoxifen.-

Principal Investigator: LI, LIAN
Grant Number: 1R01NS047199-01
Title: Characterization of a neuronal ubiquitination machinery

Abstract: Protein ubiquitination has emerged as a crucial mechanism for controlling development and function of neuronal circuits, and its defective regulation has been implicated in the pathogenesis of a variety of neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis. However, very little is presently known about the molecular machinery that controls protein ubiquitination in neurons. In the ubiquitin-proteasome pathway, substrate proteins are marked for degradation in the proteasome by covalent linkage to ubiquitin, a 76-amino acid polypeptide. The ubiquitination process involves a highly specific enzyme cascade in which ubiquitin is first activated by an E1 ubiquitin-activating enzyme, then transferred to an E2 ubiquitin-conjugating enzyme, and finally ligated to the substrate by an E3 ubiquitin-protein ligase. Of these enzymes, E3 ligase is the most important player because it determines the specificity of ubiquitin-mediated protein degradation. The importance of E3 ligases in neurodegenerative disorders is highlighted by recent findings that mutations in the E3 ligase parkin are responsible for a familial form of Parkinson's disease. In a search for neuronal proteins that regulate the neurotransmitter release machinery component SNAP-25, the applicant has discovered a novel protein, called Spring. Spring is a neuron-specific member of the RING-B-box-coiled-coil (RBCC) protein family. The importance of the RBCC family is underscored by the identification of the mutations in several RBCC proteins as the causes for a number of human diseases, including Opitz syndrome, Mulibrey nanism, and familial Mediterranean fever. In this project, the applicant will use a combination of biochemical, proteomic, molecular biological, and cell biological approaches to test the hypothesis that Spring functions as a novel E3 ubiquitin-protein ligase to regulate the turnover of the neurotransmitter release machinery. In addition, this project will characterize neuronal distribution and synaptic localization of Spring, and explore the possible involvement of this novel protein in Alzheimer's disease and Parkinson's disease. Successful completion of proposed studies will yield novel insights into the molecular mechanisms that control neuronal protein ubiquitination and neurotransmitter release, and provide fundamental information towards our ultimate goal of understanding and treating numerous neurological diseases and psychiatric disorders.-

Principal Investigator: LI, SENLIN
Grant Number: 1R01NS046004-01A1
Title: Macrophage Gene Therapy of Neurodegenerative Diseases

Abstract: Neurodegenerative diseases affect a large population of patients. Existing therapies are not satisfactory. Gene therapy holds promise, but focal delivery of DNA and the level of gene expression are challenging. Macrophages are recruited from bone marrow to most tissues of the body including the CNS, thus making them an attractive option for gene delivery. Galactosialidosis (GS) has been corrected by bone marrow-derived macrophages expressing human protective protein/cathepsin A (PPCA) transgene in a mouse model (PPCA-/-). However, correction in the CNS was incomplete due in part to weakness of the CSF-1R promoter used in the study. We have developed a series of super macrophage promoters (SMP) that are up to l00-fold stronger in vitro than the CSF-1R promoter. In models of the highly prevalent Parkinson's disease (PD), local delivery of glial cell line-derived neurotrophic factor (GDNF) has been found beneficial. We hypothesize that highly effective CNS delivery of GDNF can be achieved with the use of our super macrophage promoters and this will greatly ameliorate the pathologic changes and neurological defects in animal models of PD. To explore this hypothesis, our specific aims are: 1) To characterize these super macrophage promoters by transplantation of bone marrow stem cells transduced ex vivo with lentiviral vectors and in transgenic mice using EGFP (enhanced green fluorescent protein) as a reporter. Promoters with the greatest strength and tissue-specificity for macrophages will be used in the subsequent aims. 2) To ameliorate neurodegeneration in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of Parkinson's disease by syngeneic transplantation of HSC transduced ex vivo with lentivectors expressing GDNF gene in macrophages/macroglia driven by the SMP. Bone marrow stem cells will be transduced ex vivo with GDNF expressing lentivirus and transplanted into lethally irradiated recipient mice. Four weeks after bone marrow transplantation, the recipient mice will be injected subcutaneously with MPTP. At selected time points post MPTP administration, PET scan and behavioral testing will be performed, and brain tissue will be examined for dopamine uptake and expression of tyrosine hydroxylase (TH). In the substantia nigra pars compacta (SN), dopaminergic neurons will be counted and cell apoptosis will be assessed by TUNEL staining and immunohistochemistry for active easpase-3. 3) To ameliorate neurodegeneration in the same way as in Aim 2, but GDNF expression will be controlled by a tetracycline-regulatable gene expression system. To evaluate the effects of macrophage/ super promoter-mediated delivery and expression of GDNF on degenerating

Principal Investigator: LING, ZAODUNG
Grant Number: 5R01NS045316-02
Title: Prenatal Endotoxin as a Model of Parkinson's Disease

Abstract: Unavailable

Principal Investigator: LIU, YICHIN
Grant Number: 5F32NS042415-03
Title: Ubiquitylation of alpha-synuclein by UCH-L1

Abstract: Parkinson?s disease (PD) is characterized by the presence of Lewy bodies, the cytoplasmic neuronal inclusions, in the substantia nigra. One major component of the Lewy bodies was identified to be the fibril a-synuclein, thus linked the accumulation of this protein to the pathogenesis of PD. Failure to regulate the concentration of a-synuclein can contribute to the build-up and consequently fibrillization of the protein. The objective of this proposed research is to investigate the degradation process of a-synuclein through the ubiquitin/proteasome pathway. Neuronal ubiquitin C-terminal hydrolase (UCH-L1) hydrolyzes C-terminal ester and amides of ubiquitin and is believed to play a key role in processing polyubiquitin and/or ubiquitylated proteolytic peptide. A mutation (I93M) of the enzyme has been linked to a rare early-onset form of PD, at the same time a polymorphism of the enzyme (S18Y) was indicated to reduce the risk of PD. By direct comparing the specific activities of the wild-type and mutant UCH-L1 enzymes using techniques in biochemistry, biophysics and molecular biology, we hope we will have a better understanding of the degradation pathway of a-synuclein and the role of the UCH-L1 in the proteasome.-

Principal Investigator: LOUIS, ELAN D
Grant Number: 5R01NS042859-02
Title: Pathogenesis Of Essential Tremor: Cerebellar Metabolism

Abstract: Essential tremor (ET) is the most common tremor disorder, twenty times more prevalent than Parkinson's disease. Up to 6% of the general population has ET. Uncontrollable trembling eventually forces 10 - 25% of patients to retire prematurely. There is no cure, and few medications lessen the tremor, although deep brain stimulation has provided promising results. Clinical evidence and neuro-imaging studies suggest that the cerebellum is centrally involved in ET, and evidence from clinical and animal studies suggests that there may be a disturbance in the gamma amino butyric acid (GABA) neurotransmitter system. While ET is clinically progressive, little is known about its underlying pathology. There have been few published postmortem examinations. The fundamental question in ET research is whether an underlying pathology can be identified in terms of morphological or morphometric changes of specific cell types in specific brain regions? Second, is there a neurotransmitter abnormality in ET, either resulting as a consequence of cell loss or in the absence of cell loss? The proposed study will be a collaborative effort involving four centers in the United States and Canada where archival postmortem tissue on 24 ET patients is available. In addition, with the help of the International Essential Tremor Foundation, we will establish at Columbia University a centralized repository for new prospectively-collected ET brains, collecting 36 additional ET brains during the five-year period. The 60 ET brains will be compared with 40 control brains. Primary Aim 1 is to study the pathology of ET to determine whether there are changes in specific brain regions. Using conventional morphological methods and quantitative morphometric assessments (stereology), tissue will be examined for changes, including cell loss, in the main region of interest (cerebellar hemispheres) and in secondary regions of interest (red nuclei, thalami, inferior olivary nuclei). We hypothesize that changes and cell loss in the cerebellum will be present to a greater extent in ET than in control brains. Primary Aim 2 is to study the GABA neurotransmitter system. We hypothesize that there will be differences in cerebellar GABA-ergic immuno-labeling in ET compared to control brains. Current therapies for ET have come to us by serendipity and are ineffective in up to 50% of patients. Knowledge of the pathological changes and neurochemical abnormality in ET is critical for the design of new therapies for ET.-

Principal Investigator: LOW, PHILLIP A
Grant Number: 5P50NS032352-10
Title: AUTONOMIC DISORDERS PROGRAM PROJECT

Abstract: Autonomic dysfunction including orthostatic hypertension (OH) is a major health problem, causing significant morbidity and mortality. Its pathophysiology remains poorly understood and hence its management lacks a solid scientific base. The PPG focuses on the pathophysiology and treatment of autonomic failure. Project 1 (Low) incorporates a novel strategy of cholinesterase inhibition in the treatment of OH, an approach that promises to improve OH without supine hypertension. A second blinded treatment trial will evaluate if sodium chloride will expand plasma volume and if urinary sodium excretion is a suitable surrogate measure of plasma volume status. A series of studies, including the use of microneurography to measure sympathetic impuls3es, will evaluate the pathophysiology of postural tachycardia syndrome (POTS). A novel approach of amplitude modulation of the EEG in POTS shows a selective reduction of a frequency band of 0.02-0.05 Hz; this component is of particular interest since it may have a brainstem origin. The venous capacitance bed will be evaluated (Projects) to determine if there is excessive transcapillary efflux and changes in compliance in POTS and the effects of aging. The relative importance of the mesenteric, systematic and cerebrovascular circulations in OH will be evaluated. Project (Benarroch) will expand its studies on the neurochemical organization of autonomic control regions of the medulla in multiple system atrophy (MSA) and the parkinsonian syndromes. These include quantitative evaluates of new cellular groups (nucleus ambiguus, nuclease retroambiguus) and new receptors (including angiotensin II) that are likely to provide insights into the pathophysiology of autonomic failure in MSA. Project (Joyner) will undertake a detailed evaluation of the effects of denervation (mild in POTS and severe in neurogenic OH) and aging on the venous capacity and compliance. Project (Brimijoin) will focus on the response of the pre-ganglionic neuron to denervation and will study the mechanism of spinal intermediolateral column cell loss, using he model of immune-mediated pre-ganglionic autonomic neuropathy. The roles of apoptosis, excitotoxicity, growth factors, and aging will be evaluated and related to MSA. -

Principal Investigator: LYNCH, WILLIAM P
Grant Number: 5R01NS037614-07
Title: MICROGLIA IN RETROVIRUS INDUCED NEURODEGENERATION

Abstract: Several classes of murine leukemia viruses (MLVs) are capable of causing progressive, non-inflammatory neurodegenerative changes in the motor system from the cerebral cortex through the spinal cord upon infection of the central nervous system (CNS). The disease presentations induced by these neurovirulent MLVs are clinically and histopathologically similar to those observed in prion-associated spongiform encephalopathies and amyotrophic lateral sclerosis (ALS). Furthermore, the neurovirulent MLV infections bear a striking resemblance to HIV-associated AIDS dementia in that the major CNS target cells are the microglia, and it is the infection of this cell type that is responsible for the precipitation of motor neuron pathology. Thus, given the similarities with human diseases, the MLV-induced neurodegenerative disease models provide appealing and accessible systems for dissecting complex issues associated with microglial involvement in neurodegeneration at the cellular and molecular levels. While several lines of evidence have clearly established that the infection of microglia by neurovirulent MLVs is the formative event in neurodegeneration, the mechanisms responsible are not yet known. Thus, the overall goal of this grant is to understand how MLV infection of microglia leads to neurodegeneration of motor system neurons. To understand the process we have subdivided the goal into two major areas of inquiry. The first area focuses on the viruses themselves and their expression within microglia and endeavors to ask "What virus features and life cycle events are required for inducing neurodegeneration?" The second area of inquiry asks "How does NV MLV infection of microglia affect their constitutive CNS function?" The expectation is that the insights gained from investigating MLV-microglial interactions will have broad applicability toward understanding the role microglia play in CNS health and disease. -

Principal Investigator: MAILMAN, RICHARD B
Grant Number: 5R01NS039036-05
Title: MOLECULAR REGULATION OF D1 DOPAMINE RECEPTOR FUNCTION

Abstract: (Adapted from applicant's abstract): This is a revised application based on data showing that the first full D1 dopamine agonist dihydrexidine that we developed caused profound acute antiparkinsonian effects in primates. Recent data indicate that some, but not all, full D1 agonists produce marked tolerance when administered repeatedly. We also have shown that D1 dopamine receptor agonists of similar efficacy can differ dramatically in desensitization liability. Using a series of rigid Dl agonists, we shall determine if selective conformations are promoted by different full Dl agonists, and whether these conformations represent different, if overlapping, receptor states compared to those that promote G protein coupling. First, we shall study differences in how novel D1 agonists functionally desensitize hemagglutinin (HA)-tagged human D1 receptors (HA-D1R) in C-6 glioma cells as affected by the extent and/or pattern of GRK activity. The rate and extent of D1 receptor phosphorylation by different agonists, and the effects of dominant negative GRK mutants, will be determined. Molecular and pharmacological tools will be used to assess the relative roles of endogenous GRKs and PKA in such desensitization. We also shall determine the cellular response of GRK to agonist exposure by assessing its translocation and interaction with G-beta-gamma complements. Second, we shall map the phosphorylated residues of the hD1R receptor. Patterns of agonist-induced receptor phosphorylation induced by GRKs and second-messenger-kinases (e.g., PKA) will be assessed by mutating subsets of serines and threonines in HA-hDl-C-6 cells. We also shall overexpress HA-D1R with and without various GRKs in HEK293 cells. The phosphorylated HA-hD1 receptor will be isolated by immunoprecipitation, cleaved with protease, and sequenced by MALDVToF, Ion Trap, and/or nano-ESI-mass spectrometry. Finally, we shall examine the relationship between GRK activity and arrestin binding in the initiation of G protein uncoupling and functional desensitization in the HA-hD1 C-6 system. The complement of Q-arrestins will be determined, and alterations in high affinity binding of GRKs and arrestins to D1 receptors measured following exposure to select D1 agonists. The loss of receptor-G-protein coupling from binding of labeled GTP-analogs will be assessed to correlate receptor uncoupling with levels of)-arrestin binding. The necessity of 5-arrestin(s) in functional desensitization of the HA-hD1 receptor in C-6 cells will be studied using dominant-negative mutants, and the relation between GRK and p-arrestins assessed using tools developed in Aim 1 studies. These studies of beta-arrestin binding and recruitment will provide another functional

Principal Investigator: MAO, ZIXU
Grant Number: 1R01NS048254-01
Title: Nuclear mechanisms of Cdk5-mediated neuronal apoptosis

Abstract: Many adult human illnesses including Alzheimer's disease (AD), and Parkinson's disease and amyotrophic lateral sclerosis (ALS) involve pathologic change of neurons, which results in their loss through apoptosis. The long-term objective of this research in our laboratory is to understand how signal-controlled intracellular mechanisms regulate neuronal survival and apoptosis during development and neurodegeneration. Studies have shown that cyclin dependent kinase 5 (Cdk5) plays a key role in the apoptosis of mature neurons. Our recent findings suggest that Cdk5 functions in the nucleus to regulate apoptosis. We propose in the present application to identify novel nuclear mechanisms by which Cdk5 induces neuronal apoptosis. Our specific aims are: 1. to identify and characterize novel regulatory targets of Cdk5 in neuronal nucleus; 2. to explore regulation of p38 MAPK signaling pathway by Cdk5 in neurons; and 3. to identify and characterize additional Cdk5 regulatory sites in MEF2 (myocyte enhancer factor 2). To identify novel nuclear substrates of Cdk5, we will use Cdk5 binding assays to first isolate proteins from nuclear preparations of primary neurons and reveal their identify by Mass phosphorylation by mutagenesis. We will assess how Cdk5-mediated phosphorylation of p38 MAPK affects its subcellular localization and Spectrometer analysis. We will then confirm them as Cdk5 substrates in biochemical and biological assays. To explore whether Cdk5 directly regulates p38 MAPK pathway, we will test if p38 MAPK is a substrate of Cdk5 by in vitro kinase assays and in vivo phosphorylation studies. We will determine sites of regulation of its downstream targets. We will test Cdk5-mediated phosphorylation of p38 MAPK in models of neurotoxin-induced apoptosis. We will also explore whether Cdk5 directly regulates p38 MAPK upstream activators. Additional regulatory sites in various isoforms of MEF2 will be identified by mutagenesis. The potential effects of Cdk5-mediated phosphorylation of alternatively spliced MEF2C variants will be assessed using MEF2C mutants in reporter gene activation assays and neuronal survival assays. Identifying these novel nuclear regulatory targets of Cdk5 will allow us to explore the mechanisms by which Cdk5 regulates the survival machinery in the nucleus. This should further our understanding of the molecular process of neurotoxin-induced apoptosis, which underlies the pathogenesis of many neurodegenerative diseases -

Principal Investigator: MARAGOS, WILLIAM F
Grant Number: 5R01NS042111-04
Title: Dopamine Toxicity in Models of Huntington's Disease

Abstract: The overall goal of this application is to elucidate the roles of dopamine and the enzyme monoamine oxidase in models of Huntington's disease [HD]. Huntington's disease is a progressive neurodegenerative disorder, the pathogenesis of which is [not] completely understood. In patients with Huntington's disease, there is a mutation in the gene encoding the protein huntingtin, which results in an expanded polyglutamine sequence leading to degeneration of the basal ganglia. There is mounting evidence that metabolism of the transmitter dopamine by the enzyme monoamine oxidase, in cells in which an underlying metabolic defect exists, may lead to a cascade of events resulting in neuronal dysfunction and death. Specific Aim 1. Will determine the degree to which dopamine enhances, and MAO inhibitors attenuate, parameters of oxidative stress, mitochondrial function and neuron death in neuronal culture and mice treated with the mitochondrial inhibitor 3-nitropropionic acid. The advantage of 3-nitropropionic acid is that it provides a model of Huntington's disease resulting from relatively "pure" defect in energy production. Specific Aim 2, will examine the effects of dopamine and MAO inhibitors on these same parameters in PC6 cells transfected with full- length mutant huntingtin and cells transfected with differing lengths of polyglutamine expansions. The use of these different constructs will allow the "dose-response" relationship between polyglutamine length and toxicity to be determined and the specificity of mutant huntingtin-conferred susceptibility to DA to be established. Lastly, Specific Aim 3 will determine whether dopamine enhances, and MAO inhibitors attenuate/delay, the biochemical and neuropathological as well as behavioral abnormalities and survive in transgenic mice transfected with the first exon of the huntingtin gene. The results of these studies will demonstrate the critical role that metabolism of dopamine plays in striatal neuron death in Huntington's disease and offer a potentially novel therapeutic approach for the treatment of this disorder. -

Principal Investigator: Mckay, Ronald
Grant Number: 5Z01NS002981-06
Title: Stem Cell Biology And Brain Disease

Abstract: Unavailable

Principal Investigator: Mckay, Ronald
Grant Number: 5Z01NS002881-12
Title: The Molecular Biology Of The Mammalian Brain

Abstract: Unavailable

Principal Investigator: MCMURRAY, CYNTHIA T
Grant Number: 3R01NS040738-04S1
Title: TRAFFICKING DEFECTS IN HUNTINGTONS DISEASE

Abstract: Unavailable

Principal Investigator: MCMURRAY, CYNTHIA T
Grant Number: 5R01NS040738-04
Title: TRAFFICKING DEFECTS IN HUNTINGTONS DISEASE

Abstract: It is the aim of this grant to understand defects in vesicular trafficking and cytoskeleton that may underlie Huntington's disease. Expansion of a trinucleotide repeat CAG, encoding glutamine, results in at least eight progressive neurodegenerative disorders, including Huntington's disease (HD). The mechanism by which polyglutamine expansion selectively kills neurons is largely unknown. Aggregation is generally accepted as part of pathogenesis, but it is not known whether toxicity initiates in the nucleus or the cytoplasm. Using time lapse imaging, we have tracked the localization of huntingtin in individual neurons from expression of the mutant protein to cell death. Toxicity initiates in the cytoplasm of primary neurons. Further, we have identified targets of huntingtin-mediated aggregation directly from aggregates in human brain. We show that the expanded Huntington's protein sequesters tubulin and vesicular trafficking motors into insoluble complexes. Direct video imaging of vesicles indicates that the mutant protein indeed inhibits vesicular transport particularly in the anterograde direction. The motor that is most affected appears to be kinesin and the cargo that appears most affected is mitochondria. Our data support a model for HD pathogenesis in which aggregation inhibits proteolysis of the HD protein, disrupts cytoskeletal architecture and impairs motors required for vesicular/organelle trafficking. In this proposal, we aim to test the hypothesis that sequestration of tubulin-dependent motors underlies HD regional pathology. Using gel filtration, immunoblotting and immunoprecipitation reactions, we will evaluate whether sequestration of tubulin-dependent complexes underlies HD regional pathology in human brain. By establishing primary cultures of affected and resistant neurons, we will directly test whether vesicular trafficking is altered in the presence of normal and expanded HD protein. Since mitochondria are reduced in number in the presence of the mutant huntingtin, we will monitor the fate and subcellular localization of mitochondria by confocal imaging. Alteration in transport will be correlated with the subcellular localization of the HD protein and trafficking motors using fluorescence-labeled proteins and confocal microscopy. Finally, we will refine our understanding of pathogenesis by identifying other proteins present in aggregates by mass spectrometry. The recent observations that tubulin-dependent complexes and vesicular transport may play a role in pathogenesis of ALS and Alzheimer's disease suggest that there may be common aspects to these neurological diseases.-

Principal Investigator: MCNAUGHT, KEVIN S
Grant Number: 1R01NS045999-01A1
Title: ROLE OF PROTEASOMAL DYSFUNCTION IN PARKINSON'S DISEASE

Abstract: Parkinson's disease is characterized pathologically by selective degeneration of dopamine-containing neurons in the substantia nigra pars compacta (SNc). The etiology in the vast majority of individuals with the disorder remains elusive but ageing is an important risk factor. Nigral cell death in PD is accompanied by the accumulation of oxidatively damaged proteins, aggregation of proteins and the formation of proteinaceous intracytoplasmic Lewy body inclusions. These observations suggest that failure of the ubiquitin-proteasome system (UPS), the biochemical pathway primarily responsible for the degradation of abnormal and short-lived regulatory/transcriptional proteins may underlie nigral pathology in Parkinson's disease. Indeed, mutations in the genes encoding alpha-synuclein and 2 enzymes of the UPS, namely parkin and ubiquitin Cterminal hydrolase L1, are associated with altered protein handling in rare familial forms of Parkinson's disease. However, these or similar gene defects do not occur in most patients who have sporadic Parkinson's disease. We hypothesize that defects in 26/20S proteasomes cause the UPS to fail and this underlies protein accumulation, Lewy body formation and dopaminergic neuronal death in the SNc in sporadic Parkinson's disease. Consistent with this hypothesis, our preliminary findings demonstrated structural and function defects in 26/20S proteasomes, and a several-fold increase in the levels of poorly degraded/undegraded and potentially cytotoxic ubiquitinated protein substrates, in the SNc but not elsewhere in sporadic Parkinson's disease. In addition, we showed that in aged control subjects and adult rats, dopaminergic neurons of the SNc have relatively low 26/20S proteasomal activity and poor expression of the proteasome activators (PA28 and PA700) compared to other brain regions. Further, we showed that inhibition of 26/20S proteasomal function causes selective degeneration of dopaminergic neurons with the formation of alpha-synuclein/ubiquitin-immunoreactive inclusions in primary mesencephalic cultures and in rat SNc with motor dysfunction. In this project, we propose to determine (1) if and how the structure and function of proteasomes are defective in all stages of sporadic PD; (2) if low proteasomal function normally occurs in the SNc of controls as this may underlie its selective vulnerability and degeneration in PD; (3) if proteasomal dysfunction underlies Lewy body formation; and (4) if proteasomal dysfunction plays a role in nigral dopaminergic cell death in sporadic Parkinson's disease. These studies will test our hypothesis that inadequate proteasomal function underlies both vulnerability and degeneration of the SNc in sporadic Parkinson's disease.-

Principal Investigator: MEFFERT, MOLLIE K
Grant Number: 7K08NS002238-05
Title: NFKB FUNCTION IN REGULATING NEURONAL GENE EXPRESSION

Abstract: Neuronal apopotosis and degeneration is common to a wide range of severe diseases in the nervous system including traumatic brain injury, focal ischemia, Parkinsons's disease and Alzheimers disease. In addition to the control of basic physiological processes, the NF-kB family of transcription factors have emerged as major regulators of cell life and death in many systems. Since their recent discovery in,the nervous system, both pro- and anti-apoptotic actions have been attributed to NF-kB. Our proposal will address the possibility that different mechanisms of neuronal stimulation may result in differences in the time course, degree, and subunit composition of activated neuronal NF-kB. These differences, in turn, could explain contrasting physiologic functions of neuronal NFkB in disparate settings and might lead to the development of strategies to therapeutically regulate NF-kB activation. We have selected the hippocampus as our model system for studying neuronal NF-kB because it is a well-defined area of both physiological (learning and memory) as well as pathological (stroke, Alzheimers disease) neuronal function. Our studies will use hippocampal tissue from both adult mice and neonates as well as several transgenic and knockout lines. We will identify the NF-kB family members present in hippocampal neurons and define the types of stimulation which lead to their activation. We will investigate representative neurotransmitters, neurotrophins, and cell adhesion molecules for their ability to activate neuronal NF-kB using electromobility shift assays and a kB-reporter construct. In addition, we will use a fluorescently labeled NF-kB subunit to examine the subcellular localization of NF-kB and determine its ability to translocate to the nucleus from neuronal processes following different stimulation parameters. We will examine if levels of synaptic activity within the normal physiological range functionally regulate NFkB, or if significant activation occurs only in response to stressful stimuli, Can the pattern of NFkB activation encode information on the physiological versus toxic nature of a stimulus? Electrical stimulation will be used to assess the pattern of NFkB activation and kBdependent gene expression in response to stimuli of precisely varied intensity, duration, and frequency. Our investigations will contribute new knowledge by specifically examining the roles of NF-kB family members in the regulation of neuronal gene expression by physiological or pathophysiological stimuli. As an M.D.- Ph.D. specializing in the neurosciences, I hope to have a career as an independent investigator studying transcriptional regulation and the pathogenesis of central nervous system disease. -

Principal Investigator: Meredith, Gloria
Grant Number: 5R01NS041799-05
Title: Synaptic Proteins, Trophic Factors and Neurodegeneration

Abstract: One of the most fundamental questions related to the progressive nature of neurodegeneration in human disease is how neurons die. Protecting nerve cells against morphological decline and death requires blocking intrinsic factors that inhibit neural repair. In the present proposal, we offer an innovative approach to study those factors that are active in Parkinson's disease (PD) in a new mouse model that shows synaptic loss and irreversible nigrostriatal degeneration. We propose to track changes of a key synaptic protein, a-synuclein, both in its native environment at presynaptic terminals and under neurotoxic conditions, when it becomes insoluble and accumulates. We will further correlate those changes with altered neurotrophic support. We have established an animal protocol by treating C57/bl mice with a combined regimen of 10 doses of probenecid at 250mg/kg and MPTP at 25mg/kg for 5 weeks. These mice show a slow, progressive loss of nigrostriatal dopaminergic function for at least 6 months, that mimics PD, with no signs of recovery. Three weeks after drug treatment, there is a significant reduction in the number of substantia nigra (SN) cells and dramatic changes in the subsynaptic distribution and density of a-synuclein-immunoreactive terminals. These changes could signal the beginning of a chain of events that leads to cell death. In this proposal, we will focus on the progressive deterioration of dopaminergic neurons in the SN and their inputs, and present three specific aims to be addressed through a series of hypotheses. Specifically, we plan to 1) ascertain the origin and neurochemical phenotype of synapses in the SN that contain a-synuclein and to establish whether MPTP + probenecid treatment leads to their degeneration; 2) determine, in the MPTP+P model, the temporal relationships between cell death and a-synuclein-positive synapses, decline in dopamine function and behavior; and 3) ascertain whether changes in a-synuclein expression and production are precipitated by altered neurotrophic support. The overall objective of our research is to understand the relationship between the synaptic protein, a-synuclein, neurotrophic support, especially brain-derived neurotrophic factor (BDNF) and their respective roles in the PD form of neurodegeneration. The findings of this research should shed light on target areas where neuroprotection strategies can be implemented. -

Principal Investigator: METZ, GERLINDE A
Grant Number: 5R21NS043588-02
Title: Effects of Stress on Pathology of Parkinson's Disease

Abstract: Stress can have beneficial and maladaptive consequences depending on its type and duration. Stress can affect motor function and the course of neurodegenerative diseases of the motor system, however, this relationship has not been characterized yet. The proposed projects focus on a systematic investigation of the effects of stress and glucocorticoids, the major stress hormones, on motor function and neurodegeneration in Parkinson's disease (PD). The research proposal has three objectives: 1) Do stress and glucocorticoids affect motor performance? This objective is to characterize how stress or glucocorticoids affect skilled and locomotor movements. Glucocorticoid levels of rats will be manipulated and dose-dependent effects determined. It is expected that high levels of stress or glucocorticoids alter motor performance more likely than lower doses and that skilled movement are more susceptible to stress than locomotion. 2) Do glucocorticoids affect the onset and course of PD? This objective is to investigate the role of glucocorticoids in motor function and neurodegeneration in three rat models for PD, an acute and a progressive neurotoxic lesion, and a slow progressive lesion induced by an environmental toxin, used as a pesticide. Glucocorticoid levels will be manipulated and functional abilities assessed accordingly. It is expected that absence of glucocorticoids or chronically elevated levels lead to detrimental effects in the course of PD. 3) Can drug treatments or experience attenuate glucocorticoid-induced behavioral and histological aggravation in PD? This objective is to examine possible therapeutic interventions to protect the motor system and its function from harmful effects induced by elevated glucocorticoid levels. One series of experiments will investigate a pharmacological approach, which reduces glucocorticoid levels. Another series of experiments will assess the influence of environmental enrichment on the pathology of Parkinson's disease. It is expected that both treatments slow the progression of neural degeneration and diminish severity of motor symptoms. These projects will determine (1) the degree to which stress and glucocorticoids can influence motor learning, performance, and anatomy of the motor system, (2) the role of glucocortcoids in the etiology of PD and the progression of neural death and motor symptoms, and (3) which interventions can alter the influence of glucocorticoids on the course of PD. These approaches will provide the opportunity to understand factors involved in the pathology of PD and to develop novel therapeutic strategies for neurodegenerative motor disorders.-

Principal Investigator: MORGAN, JAMES I
Grant Number: 5R01NS040361-04
Title: Mechanisms of Cell Death in the Nervous System

Abstract: Programmed cell death (PCD) is a strictly regulated process and its disruption results in myriad developmental deficits and pathological sequelae. PCD is especially critical in the mammalian nervous system where its perturbation results in aberrant neural development and contributes to many neural disorders. There has been much research into the role of caspases in cell death. However, there is growing evidence for the importance of caspase-independent cell killing in the mammalian nervous system and other tissues. It is difficult to identify the components of the latter pathway or establish its contribution to cell elimination in vivo as it is intimately interwoven with, and masked by, the ubiquitous caspase cascades. We have developed a paradigm that can circumventthis limitation. CED-4S is a pro-apoptotic protein from C. elegans that is lethal when expressed in Saccharomyces cerevisiae. CED-4S lethality in yeast shows physiological specificity as it is blocked by its natural antagonist, CED-9 and is not mimicked by its anti-apoptotic splice variant, CED-4L. However, CED-4S toxicity in yeast does not require a caspase. Given the high degree of structural conservation amongst components of the cell suicide machinery, we propose to use CED-4S lethality in yeast as a paradigm to isolate molecules involved in caspase-independent killing. Subsequently, we will identify the mammalian counterparts of these molecules and investigate their function in the vertebrate nervous system. Using a CED-4S suppresser screen, we isolated 2 yeast AAA-ATPases, Cdc48 and yAPO-1 that have homologs in higher eukaryotes that have been implicated in neuronal death. Cdc48 binds to CED-4 whereas yAPO-l does not. This suggests a scenario in which CED-4S complexes with Cdc48 and alters its function, thereby leading to death. yAPO-l may have a redundant function with Cdc48 or it may lie downstream in the caspase-independent death pathway. Based upon these findings, we will use yeast and mammalian models to characterize the caspase-independent death pathway and determine the role that these and other CED-4 suppressers play in neuronal death in mice. In Specific Aim 1, we will determine the composition and functional domains of CED-4-containing complexes in yeast. In Specific Aim 2, downstream targets of CED-4 will be identified in yeast using a CED-4S suppresser screen. In Specific Aim 3, we will determine the expression and function of the mammalian homologs of the CED-4 suppressers in developing and adult brain and assess their contribution to normal and pathological cell death in the nervous system. -

Principal Investigator: MOSLEY, RODNEY L
Grant Number: 1R21NS049264-01
Title: Neuroprotective Vaccination for Parkinson's Disease

Abstract: Microglia inflammation contributes, in significant measure, to the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) during idiopathic Parkinson's disease (PD). Attenuation of such inflammation could attenuate disease. To this end we show that microglial deactivation responses, induced by vaccination, in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) intoxicated mice improves dopaminergic neuronal survival. This was achieved by adoptively transferring spleen cells from copolymer-1 (Cop-1) immunized mice to MPTP-treated recipients. Spleen cells from ovalbumin (OVA) injected mice failed to affect neuronal protection. Thus, our preliminary works show that protection from dopaminergic neurodegeneration can be achieved by adaptive immunity with T cells specific for Cop-1. Based on response kinetics, antigen specificity, and functional adaptive T cell immune responses, we predict that the mechanism(s) of neuroprotective immunity can be realized and could provide novel treatment strategies for human disease. Our hypothesis posits that protection from dopaminergic neurodegeneration by Cop-1 vaccination is generated through immune cell-mediated mechanisms with specificity for Cop-1 peptides and self-antigens. To investigate this we will adoptively transfer T lymphocytes, B cells and monocytes from Cop-1 immunized mice into MPTP-treated animals. Neuroprotection will be assessed by numbers of dopaminergic neurons, neurotransmitter levels, and neuronal metabolites by magnetic resonance spectroscopic imaging (MRSI). Immune cell populations, proven relevant to neuroprotection will be evaluated for the expression of gene products that are cell population specific as candidates for neuroprotection. Genetic fingerprint analysis will include cDNA microarray analysis and proteomics. This approach takes advantage of an integrated and well-established research program within the Center for Neurovirology and Neurodegenerative Disorders and builds upon research activities in PD supported previously through private donations. These approaches could prove useful for treatment of human PD. -

Principal Investigator: Mouradian, Maral
Grant Number: 5Z01NS002826-14
Title: Molecular Pathogenesis Of Cell Death In Neurodegenerative Diseases

Abstract: Unavailable

Principal Investigator: MUCHOWSKI, PAUL J
Grant Number: 1R01NS047237-01
Title: Modifiers of Huntingtin and Alpha-synuclein Toxicity

Abstract: Huntington's disease (HD) is an autosomal dominant inherited disorder characterized by involuntary movements, personality changes and dementia, and is caused by an expansion of a CAG/polyglutamine repeat in the IT-15 gene. A major neuropathological hallmark in HD is the occurrence of intranuclear and cytoplasmic inclusion bodies that contain huntingtin (the protein encoded by IT-15). Cytoplasmic inclusion bodies (Lewy bodies) are also a prominent nature of Parkinson's disease (PD), a neurodegenerative disorder characterized by muscle rigidity, bradykinesia, resting tremor and postural instability. Lewy bodies are composed primarily of the protein alpha-synuclein, and two point mutations in the alpha-synuclein gene cause early-onset, inherited forms of Parkinson's disease. Alpha-synuclein and huntingtin aggregate into ordered fibrillar structures with properties characteristic of amyloid. The 'amyloid hypothesis', developed originally to describe the role of beta-amyloid in Alzheimer's Disease (AD), suggests that the aggregation of proteins into an ordered fibrillar structure is causally related to aberrant protein interactions that culminate in neuronal dysfunction and cell death (Hardy and Selkoe, 2002). The precise roles of protein aggregation, amyloid formation and inclusion bodies in neurodegeneration remain controversial, and it is not yet clear if common molecular mechanisms underlie HD and Parkinson's disease. We have used yeast as a model eukaryotic organism to test the hypothesis that the downstream targets and molecular mechanisms by which huntingtin and ot-synuclein mediate toxicity are unique. Using a genome-wide screening approach in yeast we isolated 52 genes that modify huntingtin toxicity, and 86 genes that modify alpha-synuclein toxicity. 30% of genes that affect huntingtin toxicity are enriched in the functionally related categories of protein folding and cell stress, while 29% of genes that modify alpha-synuclein toxicity are involved in vesicular transport and lipid metabolism. Our preliminary results indicate surprisingly that the genes and cellular pathways that modulate huntingtin and alpha-synuclein toxicity in yeast are completely divergent. Nearly half of the genes we isolated are annotated as having one or more human ortholog, suggesting we may have discovered in yeast conserved cell-biological response pathways to huntingtin and alpha-synuclein that are relevant to HD and Parkinson's disease. Using the resources and information that we have generated, we now wish to advance our understanding of the neurodegeneration that occurs in HD and PD by applying molecular genetic and biochemical techniques to validate (or invalidate) the genetic modifiers we have identified. Our

Principal Investigator: MUZYCZKA, NICHOLAS
Grant Number: 3P01NS036302-06A1S1
Title: Adeno-Associated Virus Gene Transfer to Nervous System

Abstract: Unavailable

Principal Investigator: MUZYCZKA, NICHOLAS
Grant Number: 2P01NS036302-06A1
Title: Adeno-Associated Virus Gene Transfer to Nervous System

Abstract: The long term goal of this Program is to develop gene transfer methods for the treatment of neural disorders. Three groups that are well integrated have come together to develop methods for using recombinant Adeno-associated virus (rAAV) for the treatment of retinal and CNS neurodegenerative diseases. Project 1 (Muzyczka) proposes genetic experiments to identify the proteins in the substantia nigra and striatum that interact with alpha synuclein. It will specifically examine alpha syn interactions with GRK and PLD2, and develop for the first time somatic knockouts of GRK and PLD2 using AAV vectors. It will also examine the effect of oxidative stress in combination with alpha syn overexpression on neurodegeneration in the substantia nigra. Finally, it will use biochemical techniques to directly identify protein complexes that contain alpha syn. Project 2 (Hauswirth and Lewin) will take the next step toward developing a therapy for P23H rhodopsin RP using the ribozymes they developed in the previous grant period. Further, they will test two new strategies for RP that are likely to be of more general use for all RP diseases. The first is the use of GDNF expression to promote photoreceptor survival. The second is to replace all (wild type and mutant) endogenous rhodopsin mRNAs with a wild type mRNA. If successful, this should prove to be a general approach that could be applied to all genetic RP, regardless of the point mutant involved. Project 4 (Mandel) will extend their preclinical experiments toward developing AAV mediated gene transfer for Parkinson disease. Specifically, they will develop regulatable GDNF constructs that are a prerequisite for clinical applications, do the first comprehensive analysis of the immune response to AAV vectors that are injected into the brain, and test their therapeutic GNDF strategy in a primate model of Parkinson's to obtain dosing information and confirmation of efficacy in a brain model closer to human. Two cores are also proposed. Core A (Administration) will continue in its role of providing fiscal/administrative support, educational programs, and program oversight in the form of internal and external advisors. The Vector Core will continue to improve the efficiency and scaleability of rAAV vectors. In addition to providing the routine service of production and purification of rAAV2-based vectors, the Core will also develop methods for purification of alternative AAV serotypes and capsid mutants to be used in projects 1, 2, and 3.-

Principal Investigator: NATHANSON, NEIL M
Grant Number: 5R01NS034010-09
Title: MOLECULAR ANALYSIS OF NEUROKINE SIGNAL TRANSDUCTION

Abstract: The overall goal of this research is to delineate the mechanisms which regulate signal transduction by the neurally active cytokines, leukemia inhibitor factor (LIF) and ciliary neurotrophic factor (CNTF). LIF and CNTF use an overlapping set of receptor polypeptides: LIF action is mediated by a heterodimeric receptor consisting of the low affinity LIF receptor (LIFR) and gp130, and CNTF uses a receptor consisting of LIFR, gp130, and the (nonsignaling) low affinity CNTF receptor. LIF and CNTF are members of a family of pluripotent cytokines that can regulate both neuronal survival and phenotypic expression of neuropeptides and neurotransmitters. The specific aims of this proposal are: (l) To determine the mechanisms responsible for the regulation of LIF receptor signaling by mltogen-activated protein kinase (MAPK). We have found that the LIFR is phosphorylated by MAPK after stimulation of cells with LIF or other growth factors. Removal of the MAPK phosphorylation site eliminates the ability of heterologous receptor activation to regulate LIFR-mediated induction of gene expression. This proposal will determine the molecular and cellular mechanisms responsible for the regulation of neurokine signal transduction by MAPK-mediated phosphorylation of the LIFR. (2) To determine the functional consequences of LIF-stimulated serine phosphorylation of gp130, and to identify the kinase responsible for this phosphorylation. We have demonstrated that gp130 is rapidly serine phosphorylated by an unknown kinase after stimulation with LIF. The site of phosphorylation is adjacent to a dileucine sequence previously shown to be involved in receptor internalization. We will determine the role of this phosphorylation in LIF-mediated signal transduction and receptor internalization, and identify the protein kinase(s) responsible for this phosphorylation, (3) To determine the role of src family kinases in the action of neurokine receptor signaling in the nervous system. Previous work has demonstrated that a number of src family kinases are associated with the activated LIF receptor, and we have found that that src kinases are activated following LIF stimulation of both neuronal cell lines and cultured neurons. This proposal will determine the role of src family kinases in neurokine action in neuronal cells. The research described here should provide new information on the function and regulation of the neurokine receptors and on the molecular basis for their diverse actions in the nervous system. -

Principal Investigator: Nicholls, David
Grant Number: 5R01NS041908-04
Title: Mitochondrial control of neuronal excitotoxicity

Abstract: In the USA stroke kills 155,000 people per annum and is the third largest cause of death, after diseases of the heart and cancer. Furthermore 4,000,000 stroke survivors in the USA alone are coping with its debilitating effects. Stroke rates rise sharply with age, thus the increasing aging population will further increase its incidence. There is a brief window of opportunity in the hours following stroke during which the damage to the brain can be kept to a minimum, but the design of rational therapy would be facilitated if we had greater understanding of the underlying processes. Neuronal damage following stroke is amplified by the pathological release of glutamate (excitotoxicity), the consequent chronic activation of NMDA selective glutamate receptors and the influx of calcium into the cell. Mitochondrial calcium loading and consequent dysfunction is implicated not only in stroke but also in chronic neurodegenerative disorders such as Parkinson's and Huntington's diseases. The long term goal of this study is therefore to develop a comprehensive understanding of the acute and chronic consequences for the mitochondrion and neuron of pathological NMDA receptor activation, to use this information to devise and test neuroprotective strategies for the brief therapeutic window following stroke, and to relate these findings to the slow neurodegenerative disorders in which mitochondrial dysfunction is implicated. Mitochondria generate ATP, but also detoxify reactive oxygen species produced by the respiratory chain, control the cellular redox state and regulate cytoplasmic Ca2+. Any combination of these pathways may dysfunction in these disorders and the challenge is to unravel their complex interactions to identify primary lesions and suggest therapeutic and preventative strategies. The hypothesis that this application will test is that changes in mitochondrial bioenergetics function initiated during brief glutamate exposure continue even in the absence of receptor activation and are responsible for the delayed death of the neuron. To test this hypothesis we propose studies with the following specific aims. 1. To establish the immediate and delayed bioenergetics consequences of transient pathological NMDA receptor activation for cultured cerebellar granule neurons and their in situ mitochondria. While the granule cell in vivo is relatively resistant to excitotoxicity, the extensive existing information on the bioenergetics properties of these cells, coupled with their tolerance to a range of mitochondrial inhibitors makes the preparation suitable to establish principles applicable to neurons in general. 2. To establish the mechanism by which the initial transient exposure to glutamate initiates the subsequent mitochondrial

Principal Investigator: NOSHENY, RACHEL L
Grant Number: 5F31NS046234-02
Title: Gp120-Mediated Cell Death in the Basal Ganglia

Abstract: More than 50% of human immunodeficiency virus type 1 (HIV-1) infected individuals experience neurological and psychiatric problems that are collectively termed the AIDS Dementia Complex (ADC). The current global AIDS crisis highlights the need for therapeutic strategies to treat ADC. A wealth of experimental data has implicated glycoprotein gpl20, an HIV-derived envelope protein that facilitates viral entry into cells, in the cell death associated with ADC. Clinical observations of ADC patients, in vitro characterization of cell types vulnerable to gp 120 neurotoxicity, and preliminary in vivo data in our laboratory suggest that basal ganglia dysfunction, especially of the nigro-striatal pathway, is integral to the neurological manifestations in ADC. Neurotrophic factors are naturally occurring proteins that are essential for brain development and maintenance of neuronal populations affected in ADC. The proposed experiments will examine the hypothesis that gp 120 causes cell death in the basal ganglia and that neurotrophic factors can protect against gp 120-mediated cell death. This neuroprotection in turn may limit neurological complications associated with HIV infection in the brain. -

Principal Investigator: O'BANION, MICHAEL
Grant Number: 5R01NS033553-10
Title: IL-1nduced Mediators of CNS Inflammation and AD: PGE

Abstract: Neuroinflammation is a prominent feature of the CNS response to acute injury, infection, and chronic neurodegeneration, and represents a potential target for treating CNS disorders, including Alzheimer's disease (AD). Elaboration of-inflammatory responses depends on key molecular players that drive interactions among cells. One of these players is IL-1beta, a proinflammatory cytokine implicated in acute CNS inflammation and AD. Another player is prostaglandin E2 (PGE2) produced by the inflammation-responsive protein, cyclooxygenase-2 (COX-2). PGE2 is made in brain and can be induced by IL-1beta in astrocytes and microglia. We find that COX-2 inhibitors influence astrocyte gene responses. Interestingly, treatment of cells with COX-1 selective inhibitors shows similar effects. Moreover, we find that IL-1beta up-regulates multiple components of the PGE2 synthesizing and responsive pathway in cultured astrocytes, including two recently described PGE synthases and at least one PGE receptor. These molecules are in turn influenced by COX inhibition. Finally, we find many of the same effects in vivo using direct IL-1 beta injection. Based on these results we now propose that PGE2 synthesis and response systems are modulated at multiple, interdependent levels following stimulation with proinflammatory cytokines, providing a mechanism to shift cells to a new phenotype characterized by altered production of, and responsiveness to, inflammatory mediators. We further postulate that COX-1 activity coupled to cytosolic PGE synthase (cPGES) is required as a first phase for efficient PGE2 synthesis in cultured astrocytes and in brain. To examine these hypotheses, we will characterize the regulation of PGE2 synthetic pathways by IL-1beta, focusing on an apparent dependence for COX-1 and cPGES. In the second aim, we will investigate the influence of IL-1a on cell and tissue responses to PGE2, initially focusing on regulation and actions of the EP2 subtype of PGE2 receptor. In aim three, we will employ long-term ICV infusion of IL-1beta and transgenic mutant APP mice (Tg2576) to establish whether changes observed in our acute paradigms are relevant in chronic neuroinflammation. Together, these studies examine the role of PGE2 in acute and chronic CNS inflammation. This work will provide a clearer understanding of the mechanisms by which anti-inflammatory drugs influence AD, and may reveal new avenues for therapeutic intervention. Moreover, these studies have relevance to pathological processes occurring in head trauma, stroke, and other neurodegenerative diseases where glial activation and inflammation-related changes take place.-

Principal Investigator: O'MALLEY, KAREN L
Grant Number: 2R01NS039084-05A1
Title: Mechanisms of Neuronal Death in Parkinson's Disease

Abstract: Oxidative stress is a major factor in Parkinson's Disease (PD). Dopamine (DA) itself is easily oxidized to quinone derivatives and reactive oxygen species (ROS) that impair energy metabolism and form adducts with proteins such as upsilon-synuclein. Because pharmacological depletion of DA in animal models is confounded by non-specific peripheral and central nervous system effects, the role of DA oxidation in nigral cell death has been previously impossible to address. Thus a key unanswered hypothesis in this field is that DA oxidation is a major contributor to the death of dopaminergic neurons in PD. The proposed studies address several aspects of this hypothesis including the interaction of known environmental factors in triggering DA oxidation. Specifically, the hypothesis that the DA-releasing potential of the parkinsonism-inducing drug, MPP+, is due to its ability to exchange with DA and/or to reduce intracellular pH gradients will be addressed using newly derived mice expressing enhanced green fluorescent protein from a dopaminergic locus (TH+/eGFP). Primary cultures derived from these animals as well purified synaptosomal and vesicular preparations from dopaminergic terminal fields will be used in combination with fluorescent and radioactive probes to determine the temporal aspects of DA release, intracellular membrane changes, ROS formation, ATP loss, etc in response to toxin treatment. In addition, the hypothesis that DA oxidation contributes to the death of dopaminergic cells will be directly tested in vivo using animals genetically engineered to have different levels of DA production. Behavioral, oxidative and immunocytochemical criteria will be used to establish the role of DA in both the acute and chronic MPTP model of PD. To test whether DA depletion prevents ROS, new methodologies to detect in situ ROS will be used with a battery of antibodies directed against nitrotyrosine, nitrated alpha-synuclein, etc. to temporally evaluate ROS formation following acute or chronic MPTP administration in DA deficient and wild type animals. Taken together, the proposed studies will determine whether DA oxidation plays a central role in the death of DA synthesizing cells and provide insights impossible to obtain from standard animal models. Knowledge of the source and cascade of events surrounding DA-induced free radical formation will help answer risk-benefit controversies surrounding the use of dopamine replacement therapies as well as facilitate the development of new drugs and/or treatment strategies in the pathogenesis of PD. -

Principal Investigator: PALLANCK, LEO J
Grant Number: 1R21NS048362-01
Title: Mutational Analyses of Drosophila DJ-1 Homologs

Abstract: Parkinson's disease is a prevalent neurodegenerative disorder characterized by tremors, rigidity, and bradykinesia. These symptoms arise from the degeneration of dopaminergic neurons in the substantia nigra. The cellular and molecular mechanisms responsible for neurodegeneration in Parkinson's disease remain poorly understood, although genetic and environmental factors both appear to play contributing roles. Recently, loss-of-function mutations in DJ-1, a gene of unknown function, were found to be responsible for an autosomal recessive form of Parkinson's disease. To explore the normal biological function of DJ-1, and the mechanism by which loss of DJ-1 function results in neurodegeneration, we propose to subject a pair of highly conserved Drosophila DJ-1 homologs (designated DJ-1a and DJ-1b) to mutational analysis. DJ-1a and DJ-1b function will be perturbed using P element mutagenesis, gene-targeting and double stranded RNA interference methods. The phenotypes resulting from perturbation of these genes will be fully characterized, including an analysis of dopaminergic neuron integrity. Additionally, we will characterize the global gene expression changes resulting from loss of DJ-1a and DJ-1b function and initiate screens for genetic modifiers of the DJ-1a and DJ-1b phenotypes to elucidate the biochemical pathways in which these genes function. This work should clarify the normal cellular role of DJ-1 and provide a foundation for further hypothesis-driven investigation of DJ-1 function. -

Principal Investigator: Pant, Harish
Grant Number: 5Z01NS002725-18
Title: Protein Phosphorylation And Regulation Of Cytoskeleton In Neuronal Systems

Abstract: Unavailable

Principal Investigator: PAPA, STELLA M
Grant Number: 1R01NS045962-01A1
Title: Regulation of Motor Function in Parkinson's Disease

Abstract: Motor disturbances of Parkinson's disease are caused by a series of functional alterations in the basal ganglia that derive from dopamine denervation. The mechanisms underlying those functional alterations are not completely understood yet. Moreover, long-term levodopa therapy is usually associated with disabling motor complications, such as motor fluctuations and dyskinesias, whose pathophysiology also remains obscure. The long-term objective of this project is to elucidate the pathophysiologic mechanisms of abnormal motor behaviors in Parkinson's disease in view of developing new and specific therapeutic tools. Thus, this study is aimed: -firstly, to localize functional alterations in specific basal ganglia circuits; -secondly, to determine the glutamate regulation associated to an altered neuronal function; -finally, and based on the foregoing data, to explore new therapeutic approaches by interacting with the glutamatergic neurotransmission in a region-specific manner. Specifically this project comprises three aims: 1. To study the neuronal activity of individual basal ganglia regions by single cell recording in normal and various groups of parkinsonian monkeys (MPTP-treated primates) that exhibit different motor behaviors depending on treatment conditions (i.e.: parkinsonian state, its normalization, and drug-induced dyskinesias). 2. To study the glutamate receptor sensitivity in basal ganglia regions in relation to different motor conditions by comparing the binding of receptors across animal groups. 3. To study the glutamatergic blockade in restricted basal ganglia regions by determining its effects on neuronal activity and motor behavior. The research design includes techniques ranging from single- and multiple single- unit recording of neuronal activity, autoradiographic binding of receptors, to intracerebral administration of drugs in parkinsonian monkeys whose motor abnormalities closely resemble the human disease. This project proposes a novel approach to a comprehensive study of the abnormal motor function in Parkinson's disease. Thus, it will largely contribute to the rationale for new treatments that selectively target particular motor conditions. -

Principal Investigator: PARNG, CHUENLEI
Grant Number: 1R43NS048607-01
Title: In Vivo Screen for Neuroprotective Agents

Abstract: Aberrant apoptosis is implicated in several neurodegenerative disorders including, stroke, brain trauma, spinal cord injury, Parkinson's disease, amyotrophic lateral sclerosis (ALS), Alzheimer's and Huntington's disease. These neurodegenerative diseases are associated with high morbidity and mortality, and treatment options are limited. Agents that modulate apoptosis are a major focus of drug development efforts by biopharmaceutical companies. Assessment of drug effects in a convenient vertebrate model, prior to proceeding to evaluation in complex systems, such as mouse, can potentially streamline drug development and dramatically reduce costs. Zebrafish mutants exhibiting aberrant apoptosis in the central nervous system are an excellent animal model for studying neurodegeneration. Using a zebrafish neurodegenerative mutant line and a vital dye apoptosis assay, this Small Business Innovation Research project proposes to characterize embryogenesis and apoptotic patterning in zebrafish embryos, and to develop a rapid and effective in vivo screen for neuroprotective therapeutics.-

Principal Investigator: PATEL, MANISHA
Grant Number: 1R01NS045748-01A1
Title: Mitochondrial Aconitase and Parkinson's Disease

Abstract: The long-term goal of this proposal is to elucidate the mechanism by which mitochondrial oxidative stress produces dopaminergic neuronal death in Parkinson's Disease (PD). The precise mechanism by which mitochondrial oxidative stress, bioenergetic decline and iron overload arise and collaborate to produce age-related neuronal death in Parkinson's disease remains unclear. It is hypothesized that neuronal damage in Parkinson's disease results, in part from direct superoxide radical toxicity due to oxidative inactivation of mitochondrial aconitase. The hypothesis predicts that superoxide production, arising from Complex I inhibition or abnormal dopamine metabolism, inactivates [4Fe-4S]Z+-containing mitochondrial aconitase, resulting in loss of aconitase activity and release Fe z+ and H202. Posttranslational modification of this key TCA cycle enzyme can therefore result in an increased iron load, oxidant burden and bioenergetic decline. The presence of an iron responsive element (IRE) in the 5' untranslated region of the mitochondrial aconitase Mrna provides an additional mechanism for iron dysregulation in Parkinson's disease. The proposal will utilize human PD samples, animal models of PD (1-methyl-4-phenyl-l,2,3,6-tetrahydropyridine and 6-hydroxydopamine) and dopaminergic cell culture models in conjunction with a diversity of tools and techniques that include biochemical analyses, confocal microscopy, molecular biology and transgenic/knockout/aging mice. Specific Aim 1 will determine whether mitochondrial aconitase is inactivated in human and experimental Parkinson's disease. The influence of aging and chronic mitochondrial oxidative stress will be determined using mice deficient in MnSOD, a critical mitochondrial antioxidant. Specific Aim 2 will determine whether mitochondrial aconitase inactivation contributes to impaired iron homeostasis. Specific Aim 3 will determine whether scavenging mitochondrial superoxide using native or synthetic antioxidants (e.g. MnSOD transgenic mice or metalloporphyrins) protect against mitochondrial aconitase inactivation in a manner that correlates with decreased iron overload and neuronal death in experimental Parkinson's disease. Specific Aim 4 will determine the downstream consequences of mitochondrial aconitase inactivation in experimental Parkinson's disease. Specifically, regulation of brain mitochondrial aconitase synthesis by the 5' IRE in its mRNA, impact on the TCA cycle capacity and direct neurotoxicity of aconitase gene silencing will be examined. These studies can advance our understanding of the oxidative mechanisms of neuronal death in Parkinson's disease and suggest novel therapeutic strategies

Principal Investigator: PEREZ, RUTH G
Grant Number: 5R01NS042094-03
Title: Alpha-Synuclein & Dopamine:Implications for Parkinsonism

Abstract: Parkinson's disease (PD) is characterized by a loss of dopamine (DA) neurons in substantia nigra and an accumulation of a-synuclein in Lewy bodies, the cytoplasmic inclusions of PD. These and other observations suggest that an understanding of the role of a-synuclein in these processes will provide insights into the pathogenesis of PD. Although the function of this protein is unknown, a-synuclein is likely to be a chaperone. This is suggested by a-synuclein's native unfolded structure, its ability to interact with several key cellular proteins, and its homology to 14-3-3, a family of molecular chaperones. One of the proteins to which 14-3-3 binds is tyrosine hydroxylase (TB), the rate limiting enzyme in catecholamine synthesis. The binding of 14-3-3 to TH occurs primarily to a phosphorylated serine (Ser19) in the N-terminal regulatory domain of the enzyme. The binding of 14-3-3 to Ser19 is required to increase the enzyme's activity and this activation correlates with an increase in catecholamine synthesis. An interaction of 14-3-3 with Ser40 on TH has recently been reported. We have preliminary data suggesting that: (1) a-synuclein and TB interact with each other in brain and in a dopaminergic cell line, (2) the addition of recombinant a-synuclein inhibits TH activity in an in vitro model of TH activity, and (3) overexpression of a-synuclein in a dopaminergic cell line inhibits TH activity, TH phosphorylation, and DA synthesis. Together these data suggest a key role for a-synuclein in TB regulation and link a-synuclein with the regulation of DA synthesis, storage, and release. Thus, the overall goal of this proposal is to explore the role of a-synuclein in TH regulation and DA synthesis and its impact on dopaminergic cell function under normal and pathophysiological conditions. To achieve this goal we propose three specific aims: (1) To test the hypothesis that a-synuclein inhibition of TH activity is associated with TH Ser19 and Ser40 phosphorylation.(2) To test the hypothesis that a-synuclein-induced TH-inhibition reduces DA release and that the absence of a-synuclein increases DA synthesis and release.(3) To test the hypothesis that a loss of a-synuclein-mediated inhibition of TH is associated with ROS formation and reduced cell viability. Using a unique combination of in vitro and in vivo approaches, our studies hold promise to expand both our basic understanding of TH regulation and provide further insight into the association between a-synuclein and TH as they relate to several abnormal conditions, including PD. -

Principal Investigator: PEREZ, RUTH G
Grant Number: 5R21NS045336-02
Title: Cell-based assays for neuroprotection in parkinsonism

Abstract: Unavailable

Principal Investigator: Pozzo-Miller, Lucas D.
Grant Number: 5R01NS040593-05
Title: ACTIONS OF BDNF ON CA2+ SIGNALS IN HIPPOCAMPAL NEURONS

Abstract: (Applicant's Abstract) The spatial and temporal patterns of transient elevations of the intracellular concentration of free calcium ions within spines and dendrites are crucial for synapse development and maturation. Furthermore, synaptic plasticity is regulated by several neuromodulators acting on those patterns of intracellular Ca2+ concentration in both pre and postsynaptic compartments. Recent findings indicate that neurotrophins, including brain-derived neurotrophic factor (BDNF), are also necessary for synapse development, as well as for the induction and maintenance of long-term changes in synaptic strength. Although neurotrophins have been shown to induce Ca2+ elevations, the specific mechanisms involved, the source(s) of Ca2+ ions, and the consequences for synaptic development and plasticity are not known. Therefore, the delineation of the pre and postsynaptic mechanisms triggering those Ca2+ elevations is fundamental to the understanding of how neurotrophins modulate synaptic development and plasticity. The specific hypothesis to be tested is: BDNF enhances dendritic Ca2+ elevations during synaptic activity in hippocampal CA1 pyramidal neurons by the activation of the receptor tyrosine kinase (TrkB) signaling pathway. The regulation of the spatio-temporal patterns of dendritic Ca2+ elevations by BDNF is the most likely mechanism for its modulation of synaptic development and plasticity. The fundamental information gained from these experiments will integrate the role of BDNF on synaptic maturation and plasticity with the requirement of NMDA receptors and compartmentalized dendritic Ca2+ signals necessary for the induction of long-term synaptic changes in the hippocampus. The hippocampus is one of the most susceptible cortical regions to neurodegenerative diseases. Due to its role in explicit learning and memory, severe impairments in cognitive performance occur in patients suffering neurodegenerative diseases involving hippocampal areas innervated by cholinergic systems. Neurotrophins have been implicated in the maintenance of neuronal viability in adulthood, possibly underlying the reported neuroprotection and restoration of impaired brain function in neurodegenerative disorders, such as Alzheimer's disease. These neuroprotective effects have prompted significant research on their potential clinical use as therapeutic agents. Understanding the role of neurotrophins in synapse formation, maintenance and plasticity will make fundamental contributions to the development of therapeutic strategies for the improvement of cognitive functions in certain neurodegenerative diseases, such as Alzheimer's disease. -

Principal Investigator: QUIK, MARYKA
Grant Number: 1R01NS047162-01
Title: Nicotinic and neuroprotection in a parkinson mouse model

Abstract: Our goal is to understand the effects of nigrostriatal damage and nicotine treatment on nicotinic receptor (nAChR) subtypes in the basal ganglia, and determine the relationship of any changes to neuroprotection. The rationale for such work is based, in part, on epidemiological studies showing that there is a decreased incidence of Parkinson's disease (PD) in smokers. This apparent neuroprotection may be due to nicotine in tobacco since nicotine protects against nigrostriatal damage in various experimental models. Nicotine exerts its effects by stimulating nAChRs. We hypothesize that nicotine-mediated protection against nigrostriatal damage occurs as a consequence of changes in nAChR subtypes. Our preliminary data show that there are differential changes in nAChRs subtypes and their function after MPTP treatment. In this proposal, we will test the effects of nicotine to modulate nAChRs, study its neuroprotective effects against nigrostriatal degeneration and investigate its mechanism(s) of action. This will be approached through the following Specific Aims. (1) We will test the hypothesis that nicotine administration influences nAChR expression and function in MPTP-treated mice. Although nicotine exposure is well-known to upregulate nAChRs in control animals, studies to determine its effects after nigrostriatal damage remain to be done. Next (2) we will test the hypothesis that nicotine-induced changes in nAChRs correlate with neuroprotection against nigrostriatal damage by measuring various markers of striatal dopaminergic function. These data will be correlated to changes in nAChRs to determine whether receptor alterations are linked to neuroprotection. (3) To determine whether specific nicotinic receptor subtypes are involved we will we will study whether nicotine protects against nigrostriatal damage in nAChR knockout mice. (4) Finally, experiments will be done to study the molecular mechanisms that mediate nicotine-induced neuroprotection. We will investigate the hypothesis that trophic factors such as basic fibroblast growth factor (bFGF) and brain derived neurotrophic factor (BDNF), as well as immune mediators such as interleukin-6, are involved. These studies will enhance our knowledge of the changes in nAChR expression and function with chronic nigrostriatal damage and nicotine treatment. This may allow for the design of neuroprotective strategies for PD, a disorder for which only symptomatic treatment is currently available.-

Principal Investigator: RATAN, RAJIV R
Grant Number: 5R01NS040591-04
Title: Arginase and Regulation of Nitric Oxide Synthase in ALS

Abstract: (Adapted from applicant's abstract): Amyotrophic lateral sclerosis is a prevalent neurological disorder characterized by inexorable muscle weakness leading to death. The principal pathological finding in amyotrophic lateral sclerosis is loss of nerve cells in the anterior horns of the spinal cord, the motor nuclei of the brainstem, and the upper motor neurons of the cerebral cortex. Investigations aimed at preventing or limiting progression of amyotrophic lateral sclerosis have thus focused on the mechanisms by which neurons degenerate. A transgenic mouse model has been developed that possesses many of the pathological and clinical features of human familial and sporadic amyotrophic lateral sclerosis. As nitric oxide (NO) has been shown to mediate neuronal loss in other neurodegenerative conditions, several groups have investigated the role that NO may play in disease progression

Principal Investigator: RICHARDSON, ROBERT M
Grant Number: 5F31NS042420-04
Title: Telomerase re-expression in postmorterm CNS Progenitors.

Abstract: Transplantation strategies using CNS stem cells offer tremendous potential for replacing neuronal circuitry lost to Parkinson?s disease and other neurological disorders. The ability of CNS grafts to survive after transplant is crucial if therapeutic benefit is to be provided to a large number of patients with PD. Additionally, there must be an adequate source of donor cells. The purpose of this proposal is to insert the human telomerase gene (hTERT) into human postmortem-derived neural progenitor cells (HPCPs) in order to improve the survival of transplanted cells and ultimately increase the proliferation of desired cell types. HPCPs provide an easily accessible donor source, and ectopic expression of hTERT is a logical means by which to immortalize these cells in culture and decrease their susceptibility to apoptotic death following transplantation. Determining the effect of ectopic telomerase expression in HPCPs on population doublings, resistance to apoptosis and ability to differentiate into all CNS cell types is the crucial first step in investigating these methods. Assessing the viability, proliferation and differential fates of hTERT+ HPCPs transplanted to a 6-OHDA lesioned rat model of Parkinson?s disease, and evaluating functional recovery in transplant recipients will further characterize the extent to which these approaches may contribute to future stem cell therapy for neurological disorders. For the treatment of Parkinson? disease, a future step may include combining these methods with strategies likely to induce a dopaminergic fate among a subset of these progenitors.-

Principal Investigator: ROBERTS, JAMES L
Grant Number: 5R01NS042080-03
Title: ECM and the Differentiation/Plasticity of DA Neurons

Abstract: We have recently shown in animal models of Parkinson's disease, a progressive neurodegenerative disorder characterized by the degeneration of nigrostriatal dopaminergic neurons, that there is a robust proliferative burst of neuroprogenitor cells in response to the loss of dopamine neurons. However, these cells remain in an undifferentiated state. We have also shown that in response to the degeneration of dopamine neurons in the substantia nigra that the remaining un-injured dopamine neurons are able to compensate for the loss by extending collateral axonal fibers. However, this compensatory response becomes greatly attenuated with increasing age. Therefore, based on these previous results we propose further experiments designed to discover how to induce the differentiation of dopaminergic progenitor cells and injury-induced collateral sprouting in animal models of Parkinson's disease. Integrins are cell surface receptors involved in cell-matrix and cell-cell adhesion interactions in salt organs and play an important role in regulating cell proliferation, survival, and process outgrowth. Studies outside the nervous system indicate that an integration of signals derived from both integrin-ECM interactions and soluble growth factors are required for cellular differentiation. Thus, we hypothesize that ECM molecules expressed in the CNS in combination with growth factors act together to induce neural progenitor cell commitment and differentiation. To test our hypothesis, we propose to characterize which ECM molecules are expressed during the time in development when midbrain dopaminergic progenitors are exiting the cell cycle and making their final commitment to the dopaminergic neuronal fate. In parallel, we plan to culture these progenitor cells on different ECM substrates in combination with fibroblast growth factor-2 (FGF-2) and determine whether the fate of these cells is regulated. We further hypothesize that as the brain develops, the expression of ECM molecules required for the differentiation of neural progenitor cells into dopaminergic neurons becomes down-regulated. To test this idea we plan to culture midbrain progenitor cells on tissue slices containing the substantia nigra from either developing or mature animals. Since our overall goal is to be able to induce dopaminergic progenitor cell differentiation in mature animals, we propose to test whether the induced expression of integrins by retroviral expression vector infection of uncommitted progenitors will induce their differentiation. Finally, we plan to test the hypothesis that age-related differences in ECM molecules play a role in supporting or inhibiting collateral sprouting. Thus, we plan to culture embryonic dopamine neurons on striatal tissue slices collected

Principal Investigator: RODRIGUEZ, ALICE L
Grant Number: 1F32NS049865-01
Title: Development of allosteric potentiators of mGluR4

Abstract: Treatment of Parkinson's disease (PD) has traditionally focused on dopamine replacement strategies such as LDOPA. While generally effective early on, L-DOPA has often proven inadequate for long term treatment due to serious adverse side effects. Recent studies in Dr. Conn's laboratory suggest that activators of metabotropic glutamate receptor mGluR4 may provide a novel pharmacological approach to the treatment of PD by targeting the indirect pathway of the basal ganglia. Furthermore, Dr. Conn and coworkers have developed a novel approach to activation of mGluR4 by development of allosteric potentiators that do not activate this receptor directly but dramatically potentiate the response to glutamate. While these studies provide an exciting proof of principle for a novel approach to activation of mGluR4, there is a need to develop novel compounds that have a higher potency and are useful for further in vivo studies. The goal of this work is to develop novel potent and selective allosteric potentiators of mGluR4. A threefold approach will be implemented, beginning with performing a high throughput screen mining for compounds that potentiate the glutamate response of mGluR4. In parallel with the HTS, medicinal chemistry studies will be pursued to improve upon the properties of known potentiators. Finally, mutagenesis studies will be performed to develop a better understanding of the molecular interactions involved in potentiator binding which will subsequently aid in the design of future compounds. Together these approaches will result in the development of novel small molecules that have a therapeutic effect on PD by reducing transmission through the indirect pathway. Furthermore, these studies will be complemented by ongoing electrophysiology and behavioral studies in Dr. Conn's laboratory that will determine the effects of these compounds in vitro models of basal ganglia function. -

Principal Investigator: RON, DAVID
Grant Number: 3R21NS043628-02S1
Title: Endoplasmic Reticulum Stress and Parkinson's Disease

Abstract: Unavailable

Principal Investigator: ROSS, DAVID
Grant Number: 5R01NS044613-03
Title: NQ01, Oxidative Stress and Proteasomal Inhibition

Abstract: Increased oxidative stress in dopaminergic neurons has been implicated as a causative factor in Parkinson's disease (PD). The catechols DOPA dopamine and DOPAC generated in these cells can undergo oxidation to produce aggressive oxygen species and reactive arylating quinones which are capable of cellular damage. We have characterized a null polymorphism in the NQO1 gene (NQO1 *2) and individuals carrying the variant NQO1 *2 allele have been found to be at a markedly increased risk of PD relative to control individuals emphasizing the role of NQO1 as a risk factor for PD. PD has also been associated with inhibition of the ubiquitin-proteasomal system (UPS). Inhibition of the UPS by aggregated proteins in neurodegenerative disease leads to increased oxidative stress. Since oxidative stress can result in the misfolding and aggregation of proteins resulting in further frustration of the UPS, a picture is beginning to emerge where both oxidative stress and UPS inhibition are part of a vicious cycle and are important factors in the etiology of PD.In this proposal, we will test the following hypotheses; 1) that NQO1 protects cells against reactive arylating quinones and oxidizing species generated during metabolism of DOPA, dopamine and DOPAC. We will use dopaminergic cells, mechanism based inhibitors of NQO1 and an isogenic cellular model developed in our lab to explore the role of NQOl in these studies; 2) that DOPA, dopamine or DOPAC derived o-quinones contribute to UPS inhibition and that NQO1 protects against this inhibitory effect; 3) that NQO1 protects against oxidative stress generated as a result of inhibition of the UPS. UPS inhibition in cells will be achieved by the use of both chemical proteasome inhibitors and by transfection of mutant forms of alpha-synuclein which have been associated with PD and inhibit the UPS by mechanisms involving protein aggregation and 4) that mutant NQO1*2 protein, which is normally rapidly degraded by the UPS, generates oxygen radicals leading to increased oxidative stress. The ability of mutant NQO1 *2 protein to generate oxygen radicals becomes particularly important under conditions where the UPS is inhibited and the protein accumulates.-

Principal Investigator: RUOHO, ARNOLD E
Grant Number: 5R01NS033650-09
Title: Characterization of Vesicular Monoamine Transporters

Abstract: The strategy of this proposal is based on the rationale that identification of the inhibitor, substrate, proton translocation, and functionally relevant phosphorylation sites on monoamine transporters (VMAT2) will provide a basic understanding of the mechanism of action of monoamine sequestration into vesicles and the factors which regulate transporter activity. This work will be accomplished in three Specific Aims: (1) Identification of the reserpine binding site(s) on VMAT2. Novel reserpine photoaffinity labels will be synthesized and characterized, and photo-labelled peptides will be identified in order to map the reserpine binding site; (2) Identification of the substrate transport channel. This aim will involve the use of several approaches, including radioactive photo-activatable substrate analogs to covalently derivatize the substrate binding site on VMAT2; site-specific derivatization of VMAT2 at engineered cysteine residues with the cysteine-reactive reagents, methanethiosulfonate ethyl amine (MTSEA), and MTS-ethyltrimethylammonium (MTSET); and site-directed mutagenesis of potential residues lining the channel; (3) Determination of the functional role of two highly charged regions of VMAT2. This aim will involve the use of biochemical and genetic (site-directed mutagenesis) approaches to determine the role of phosphorylation of the N-terminus of VMAT2 on transporter function and the intracellular distribution/oligomeric state of the transporter. Reduced or aberrant activity of the monoamine transporter of the synaptic vesicles in dopaminergic neurons of the substantia nigra through either direct or indirect actions of toxicants (e.g., MPP+, insecticides) and genetically altered neuronally expressed proteins may play a central role in Parkinson's Disease. The regulation of uptake of monoamine neurotransmitters into storage vesicles may also play an important role in affective psychological disorders related to depression by altering levels of serotonin, norepinephrine, dopamine, or other neurotransmitters. This work will provide insight into the mechanism of action of the monoamine transporters and contribute to our understanding of how pharmacological and therapeutic strategies may be devised to treat Parkinsonism or other disorders of the nervous system. -

Principal Investigator: SALAMONE, JOHN
Grant Number: 1R01NS047261-01
Title: Dopamine D2 and Adenosine A2A roles:Tremulous Movements

Abstract: Symptoms of parkinsonism, such as akinesia, bradykinesia, and tremor, can be caused by degeneration of dopamine (DA) neurons, or by administration of DA antagonist drugs. Parkinsonism is characterized by a cascade of neurochemical events that reflect interactions between several neurotransmitters in the circuitry of the basal ganglia, including DA, acetylcholine, serotonin, GABA and adenosine. Within the last few years, increasing evidence has accumulated indicating that central adenosine neurons play an important role in modulating the functional circuitry of the basal ganglia. Several subtypes of adenosine receptors are involved in motor function, and anatomical studies have demonstrated that the adensonine A2A receptor subtype has a relatively high degree of expression within the striatum. Although several types of striatal cells contain some adensonine A2A receptors, these receptors are present in very high densities on striatopallidal neurons, which also tend to co-express DA D2 receptors and enkephalin. It has been suggested that antagonists of adenosine A2A receptors could have some potential utility as antiparkinsonian drugs. In a recent study from our laboratory, it was demonstrated that IP injections of the adenosine A2A antagonist, KF17837, also suppressed haloperidol-induced tremulous jaw movements, and reversed the locomotor suppression induced by this D2 antagonist. This profile of activity is consistent with the hypothesis that antagonism of adenosine A2A receptors can result in antiparkinsonian effects in animal models. The proposed experiments are designed to investigate the role of the striatopallidal GABAergic pathway as a possible mediator of the putative antiparkinsonian effects of adenosine A2A antagonists. These proposed studies will focus on the tremulous jaw movement model, which is related to parkinsonian tremor. It is hypothesized that adenosine A2A antagonists are acting on striatopallidal GABAergic neurons that also express DA D2 receptors. In view of research showing that haloperidol increases extracellular GABA in globus pallidus, and that haloperidol-induced tremulous jaw movements are reduced by pallidal injections of bicuculline, it is hypothesized that doses of adenosine A2A antagonists that reduce jaw movement activity also will reduce haloperidol-induced increases in GABA release in globus pallidus. In addition, it is hypothesized that adenosine agonists and antagonists will interact to regulate the behavioral and neurochemical effects of haloperidol. These hypotheses will be investigated using studies that involve both systemic and intrastriatal injections of drugs that act upon A2A receptors, and the proposed work will involve a

Principal Investigator: SALVESEN, GUY S.
Grant Number: 5R01NS037878-06
Title: Proteases in Neuronal Cell Death

Abstract: The basic mechanisms that underlie neurodegenerative diseases are unknown. Loss of function of specific regions of the brain is due to incapacitation of cells that constitute the specialized regions. Cells can simply stop functioning normally - neurons may cease to transmit signals - or they may die. There is now evidence that the pathology of several neurodegenerative diseases is due to inappropriate cell death, specifically, adventitious activation of apoptosis. This being the case, an understanding of the mediators of apoptosis, their identities and their role in orchestrating death, would be a vital step towards remedying the diseases. The apoptotic system is mediated by proteolytic enzymes that are responsible for conducting faithful execution of all responsive human cells. We propose to study neuronal apoptosis, and the proteolytic systems that contribute to it, in a simplified model that can be manipulated to answer basic issues of cell death pathways. The object of this proposal is to understand fundamental processes of cell death in cell culture models of human neurons and in primary rodent neurons. Specifically, we will focus on the pathways to apoptosis involving caspase family proteases in cell-free models of neuronal differentiation based on the neuronal-derived SHSY5Y cell line. We will use the knowledge gathered from these studies to examine apoptosis in rodent models of neuronal apoptosis, and specifically test the hypothesis that neuronal plasticity is in part due to activation of a localized apoptotic, protease-mediated program. -

Principal Investigator: SARANG, SATINDER S
Grant Number: 1R43NS050920-01
Title: PESTICIDE-SYNUCLEIN INTERACTIONS AS RISK FACTORS FOR PD

Abstract: Parkinson's disease (PD) and other age-associated neurological disorders represent one of the largest unmet medical needs in developed countries. However, the discovery of improved diagnostics and therapeutics for these disorders is hampered by incomplete understanding of underlying disease mechanisms and risk factors. Oxidative stress, mitochondrial dysfunction, and protein aggregation have been implicated as major mechanisms causing dopaminergic neuronal loss in PD. Epidemiological studies have revealed an association between pesticide exposure and PD, and pesticides that cause oxidative stress and mitochondrial dysfunction, such as rotenone and paraquat, are used in cellular and animal models of PD. Furthermore, interactions between pesticides and the PD-linked gene alpha-synuclein have been postulated. Although almost 1000 pesticide active ingredients are currently marketed, these compounds have not been systematically screened for neurotoxicity in cellular or animal models of PD. The identification of pesticides that interact with alpha-synuclein to cause neurodegeneration may lead to the discovery of novel candidate risk factors and more representative disease models for PD. For this proposal, investigators at Cambria Biosciences will exploit a published moderate-to-high throughput neuronal cell-based model of PD, with the goal of identifying individual pesticides and synergistic pesticide combinations potentially involved in the pathogenesis of PD. Our established cellbased model of PD will be used to screen -approximately 350 registered pesticides to identify neurotoxic pesticides. Our specific aims include: (1a) identifying neurally-active pesticides that induce cell injury to two PD-like cell lines that stably express wild type (WT) human alpha-synuclein and mutant A53T alpha-synuclein; (1b) identifying any synergistic effects of neurotoxic pesticides in inducing cell damage in these a-synuclein-expressing neuronal cells; and (2) characterizing the activity of these neurotoxic pesticides and pesticide combinations using primary mature mesencephalic DA neurons. The identified neurotoxic pesticides will be employed in follow-on Phase II studies for the development of improved in vitro and in vivo PD models, which will ultimately be used to screen for neuroprotective compounds as part of a comprehensive drug discovery program. -

Principal Investigator: SCHLOSSMACHER,
Grant Number: 2K08NS002127-04
Title: The Roles of a-Synuclein and Parkin in Parkinson Disease

Abstract: The pathogenesis of Parkinson disease (PD) is unknown but dopamine-induced oxidative stress, proteasomal abnormalities and mitochondrial dysfunction are associated with its neurodegeneration. Rare heritable forms of PD are linked to an increasing number of gene loci. At the PARK1 locus, SNCA encodes a neuronal protein, alpha-synuclein (alpha-S), that is involved in the transition of synaptic vesicles from the reserve-resting pool to the readily releasable pool in vivo and in vitro. It is linked to sporadic PD by the formation of fibrillar inclusions that contain phosphorylated alpha-S, and to autosomal dominant PD by a likely gain-of-function effect of two infrequent point mutations. The PARK2 gene encodes parkin, an E3 ubiquitin ligase. It is mutated in < 50% of all autosomal recessive PD cases by a probable loss-of-function phenomenon. In normal human brain (but not rat brain), a pool of alpha-S undergoes O-linked glycosylation, thereby generating alpha-Sp22. This glycoprotein is a substrate for parkin's E3 ligase function in vitro and accumulates in PARK2-mutant PD brain. The central hypotheses of this application state that 1) a shared pathogenetic pathway is encoded by PD-linked genes, 2) characterization of the alpha-S glycosylation in primate brain will provide insights into the pathogenesis of PD, 3) the normal function of the Parkin E3 complex is essential for the sustained survival of catecholaminergic neurons in adult human brain, and 4) the identification of the in vivo subunits of the assembled parkin E3 complex will validate reported binding partners and reveal potentially neurotoxic substrates. To this end, I have identified two Specific Aims: Aim 1: To characterize the glycosylation of alpha-S in human control brain as well as PARK1-linked PD brain and to model its biosynthesis in a cell model, and Aim 2: To biochemically purify the subunits of the Parkin E3 ligase complex from human brain, and verify them in vitro.-

Principal Investigator: SCHOR, NINA F
Grant Number: 5R01NS041297-03
Title: Antioxidant Strategies for Parkinson's Disease

Abstract: Reactive oxygen species (ROS) have been implicated in the pathogenesis of Parkinson's disease. This suggests that antioxidant strategies may be useful in the treatment and/or prevention of this neurodegenerative disorder. We have developed and implemented two models for the central movement disorder and autonomic peripheral neuropathy, respectively, associated with Parkinson's disease. We propose to use these models to design and test antioxidant strategies we have previously developed for adjunctive use with ROS-generating chemotherapeutic agents. We will further use our studies of the biochemical effects of antioxidant treatment to develop a screening test for new antioxidant agents for use in Parkinson's disease and other ROS-related disorders. Specifically, we propose to test the hypothesis that recycling antioxidants increase expression of p21 wafl/cip1,enhance binding of HIF-1 and CREB to DNA, activate NF-kappaB, prevent ROS-induced morphological apoptosis, and decrease ROS-induced membrane phospholipid and protein nitration in culture models of Parkinson's disease. We will further test recycling antioxidants for their distribution to the CNS and peripheral compartments, and use this information to test CNS-penetrating and non-CNS-penetrating agents for efficacy in the central and autonomic nervous system models, respectively, of Parkinson's disease. Finally, we will test the hypothesis that the magnitude of induced in vitro biochemical change for each drug correlates with the degree of protection from the effects of ROS in the CNS or autonomic model. This latter study will pave the way for development of an in vitro screening test for new antioxidant strategies proposed for use in Parkinson's disease. This application specifically addresses the NINDS agenda for research in Parkinson's disease in its development of in vitro screening tests for putative therapeutic agents in general and antioxidants in particular for this disease, its development of animal models for the clinical aspects of Parkinson's disease, and its potential for further elucidation of the mechanisms of ROS-induced apoptosis in the nervous system.-

Principal Investigator: Sheline, CHristian T
Grant Number: 5R01NS030337-13
Title: ZINC NEUROTOXICITY

Abstract: Glutamate receptor- and Ca2+-mediated neurotoxicity was the focus of study during past grant periods. Recently, we have begun to examine a related form of neurotoxicity, also enhanced by glutamate receptor activation but mediated by Zn2+ rather than Ca2+. Zn2+-mediated neurotoxicity likely contributes to central neuronal death after certain insults, such as transient global ischemia. Our Central Hypothesis is that extracellular Zn2+ can kill neurons by: 1) entering across the plasma membrane, largely through voltage-gated Ca2+ channels (VGCCs) in depolarized neurons; 2) increasing intracellular free Zn2+ ([Zn2+]i); 3) interfering with glycolysis, causing ATP levels to fall; 4) triggering apoptosis (at lower Zn2+ levels). The proposed experiments will test aspects of this central hypothesis in cultured murine cortical neurons, delineating mechanisms underlying Zn2+-induced neuronal death to advance efforts to develop therapeutic countermeasures that might be used to reduce brain damage after cardiac arrest. Cultured neurons will be exposed to varying concentrations of extracellular zinc for brief ("fast toxicity") or prolonged ("slow toxicity") time periods. We plan to define the relationships linking transmembrane Zn2+ influx (measured with patch-clamp and radio-isotope flux techniques), [Zn2+]I (measured with dye videomacroscopy), cellular Zn2+ content (measured with atomic absorption spectroscopy or inductively-coupled plasma spectroscopy), and cellular apoptosis (v.s. necrosis). We will also measure resultant neuronal levels of ATP, NAD+, NADH and glycolytic intermediates, mitochondrial transmembrane potential, and cytoplasmic reactive oxygen species (measured with dihydroethidium dye). Finally, we will test genetic perturbations of cellular Zn2+ homeostasis, specifically increased or decreased expression of the key plasma membrane Zn2+ transporter, ZnT-1, or the major neuronal intracellular Zn2+ binding protein, metallothionein-III, will produce the changes in vulnerability to Zn2+ neurotoxicity predicted by the central hypothesis. -

Principal Investigator: SHEN, JIE
Grant Number: 5R01NS041779-04
Title: Studies of Parkin KO Cells and Mice as PD Models

Abstract: Parkinson's disease (PD) is an age-related neurodegenerative disorder affecting approximately 5% of people over age 65. PD is characterized pathologically by the selective degeneration of dopaminergic neurons in the substantia nigra and the formation of intraneuronal inclusions known as Lewy bodies. Recessively inherited mutations in the Parkin gene are the most common cause of inherited and early onset PD. A variety of large Parkin deletion and truncation mutations as well as missense mutations have been linked to PD in many families, strongly indicating that recessively inherited parkinsonism is caused by loss of Parkin function. The central hypothesis underlying our research is that loss-of-function mutations in the Parkin gene alter the normal physiology of dopaminergic neurons in the substantia nigra, ultimately leading to the parkinsonian phenotype. A loss-of-function pathogenic mechanism can be studied in cells and animals from which the Parkin gene has been deleted. Knockout mice are commonly used to investigate the normal function of genes. Knockout mice can also be used to study diseases caused by gene deletions in humans. Parkin knockout mice can be used to study the abnormal nigral degeneration caused by loss of Parkin function in humans. To investigate the role of Parkin in the survival of dopaminergic neurons, we propose to generate mice with targeted germ-line disruption of the Parkin locus. The Parkin knockout mice will then be analyzed for biochemical and neuropathological abnormalities associated with PD, such as degeneration of dopaminergic neurons, reductions in striatal dopamine levels, and motor behavioral deficits. In parallel, we will generate and analyze Parkin knockout cells in vitro. This will provide a powerful cellular system with which to characterize the function of Parkin and to examine the consequences of its absence, such as increased sensitivity to oxidative stress and apoptotic stimuli. Both the animal and the cellular systems could provide valuable means for identifying and testing molecules and genes with therapeutic potential. -

Principal Investigator: SHERMAN, MICHAEL Y
Grant Number: 1R01NS047705-01A1
Title: Cell Mechanisms of Abnormal Protein Aggregation

Abstract: Neuronal accumulation of various mutant or damaged proteins results in several neurodegenerative disorders, including Parkinson's disease, ALS, and polyglutamine expansion disorders. Toxic abnormal species can aggregate in cells, and there is an ongoing discussion of how protein aggregation influences neurotoxicity. It has recently become clear that in contrast to protein aggregation in a test tube, aggregation of damaged or mutant polypeptides in vivo is a complicated and tightly regulated process that involves many cellular factors. Using a yeast model of polyglutamine (polyQ) expansion disorders, the PI has carried out a number of genetic screens and found that mutations in several components of the machinery that organizes cortical actin patches (CP) dramatically reduce polyQ aggregation. Furthermore, elimination of CP by arp2 or arp3 mutations completely blocks aggregation of polyQ in cells. This proposal will test the hypothesis that cortical actin patches play a direct role in polyQ aggregation. Accordingly, investigations will be carried out to determine if polyQ-containing polypeptides aggregate at CP sites, and the role of Rnq1 prion in these interactions. The role of components of CP and factors responsible for formation of actin cables in polyQ aggregation will be evaluated. An important goal would be to establish whether CP play a general role in protein aggregation, including aggregation of distinct proteins important for neurological disorders, e.g. synphilin 1, alpha-synuclein and PABP2, and in formation of yeast prions. A critical question will be whether homologs of major components of CP play similar role in polyQ aggregation in mammalian cells. A special focus will be to evaluate a hypothesis that interactions between CP and polyQ are mediated by certain SH3-domain proteins, e.g. Sla1, Rvs167, Bem1 or Hof1. Previous work from the PI's lab showed that polyQ aggregation causes early defect in endocytosis in yeast and mammalian cells. In a separate aim we will test a hypothesis that polyQ aggregation causes inhibition of endocytosis in neurons, using a C. elegans model. It will also be established whether mutations that increase the lifespan and delay the onset of polyQ aggregation in worms also delay the onset of endocytosis defects. Exploration of fundamental mechanisms of protein aggregation that we undertake in this project will help to understand the nature of several neurological disorders. -

Principal Investigator: SIDHU, ANITA
Grant Number: 5R01NS041555-03
Title: DOPAMINERGIC SIGNALING IN NEUROLOGICAL DISORDERS

Abstract: Imbalances in dopamine (DA) receptor/G protein coupling dynamics are important in the onset and maintenance of several neuropathological diseases, such as schizophrenia, Parkinson's disease, drug abuse and attention deficit disorder. The D1-like receptors, D1 and D5, share similar structural, physiological and pharmacological homology. The functional attributes of these receptors in DA neurotransmission are largely unknown in diseased and normal states. We have shown that in transfected cells, these receptors can be functionally differentiated in that: D1 receptors couple to both G(s)alpha and G(o)alpha, while D5 couples to G(s)alpha and G(z)alpha. Moreover, D5 but not D1 receptors, inhibit phosphoinositide production. Moreover D1, but not D5, can inhibit adenyl cyclase activity, in the absence of receptor/G(s)alpha coupling. Through functional assays, we will examine the mechanism and functional consequences of D1 coupling to G(o)alpha, and D5 to G(z)alpha, in order to determine whether such coupling causes activation of alternate signaling pathways. Using progressively shorter synthetic peptides directed against specific amino acid motifs of intracellular loops of the D1 and D5 receptor, we will map the domains through which D1 couples to G(s)alpha/G(o)alpha and D5 to G(s)alpha/G(z)alpha. The ability of various peptides to block receptor/Galpha interactions will be examined through co-immunoprecipitation and functional assays. Deletion mutants will be constructed to demonstrate the participation of specific sites in receptor function. We will analyze the interactions between D1 and D5 receptors with their cognate G proteins, using a highly sensitive novel assay, fluorescence resonance energy transfer (FRET). Such FRET studies will enable us to determine in intact cells whether the receptors couple simultaneously to the two Galpha, or if such coupling occurs in a sequential manner. We will also examine interactions between synthetic peptides and G proteins, and determine whether receptor oligomerization is essential for dual coupling of D1 and D5 receptors to Galpha. A clear understanding of the mechanism and functional consequences of coupling of these receptors to different and diverse Galpha is important for defining the roles of these receptors in diseased and normal states, and may aid in the design of novel therapeutic treatments, to selectively activate or suppress specific signaling pathways. -

Principal Investigator: SIDHU, ANITA
Grant Number: 5R01NS034914-07
Title: Dopamine and Oxidative Stress in Parkinson's Disease.

Abstract: Oxidative stress is an important causative factor in the onset and maintenance of several neurodegenerative conditions, such as Alzheimer's disease and Parkinson's Disease (PD). While dopamine (DA)-replacement therapy can control the symptoms of PD, it can also cause severe dyskinesia in patients. Blockage of the D1 DA receptors with Dl-selective antagonists in 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP)-lesioned primates, significantly improves dyskinesia, through unknown mechanisms. Autoxidation of DA is a major source of free radicals; activation of D1 receptors also triggers oxidative stress, and these effects are additive, such that the resulting damage produced in the postsynaptic cell is several fold greater than that elicited by sources of free radicals (hydrogen peroxide (H202)), which does not stimulate the D1 receptor. Several indices of oxidative stress, lipid peroxidation, nitrite production, nitric oxide synthases, neurofilament (NF)-kappaB nuclear translocation, are all elevated two to six fold higher with DA-mediated D1 receptor activation, than H202 alone. We will examine in detail the contribution of D1 receptor stimulation, through the use of agonists and antagonists, in causing oxidative stress in SK-N-MC human neuroblastoma cells, which endogenously express the D1 receptor and is representative of postsynaptic cells. We will examine the mechanism and functional consequences of D1 receptor stimulation on signaling pathways, as well as by selectively blocking parts of the oxidative stress cascade(s). The participation of D1 receptors and oxidative stress in cell death and apoptosis will also be measured. Since blockage of D1 receptors in the MPTP model of PD improves some of the symptoms of PD, we will investigate whether D1 receptors augment MPTP effects in SK-N-MC cells. Conversely, blockage of D1 receptors with antagonists may attenuate MPTP effects on the various indices of oxidative stress. A clear understanding of the effects of dopamine autoxidation and the participation of D1 DA receptors in inducing oxidative stress, is important for understanding patient response to agonist therapy in PD, and may aid in the design of novel therapeutic treatments. -

Principal Investigator: SIDHU, ANITA
Grant Number: 5R01NS045326-02
Title: SYNUCLEOPATHIES IN NEURODEGENERATION

Abstract: The family of synucleins is abundantly present in presynaptic neurons and is associated with numerous neurodegenerative disease states, collectively termed synucleinopathies. One member of this family, (-synuclein, is present as the major component of Lewy bodies [LB] in LB variant of Alzheimer's disease, dementia with LBs, sporadic Parkinson's disease, multiple system atrophy and neurodegeneration with brain iron accumulation. Mutants of alpha-synuclein, the A30P and A53T alpha-synucleins, are present in LBs of certain genetic forms of PD. However, neither the primary normal function of alpha-synuclein nor its mode of disease inducing action is known. Understanding the molecular and functional correlates of alpha-synuclein would help in the understanding of both the normative, and aberrant activity of this protein that give rise to the formation of fibrillary aggregates and LBs, in a process that is accelerated by increased oxidative stress and inflammation found in diseases of the aged. We provide preliminary evidence to suggest that one possible novel role for (-synuclein is to regulate the activity of the presynaptic dopamine transporter [DAT]. This regulation of DAT activity is bimodal, causing both the increased activity of the transporter and attenuation of normal transporter activity, as is indexed by [31H] dopamine uptake assays. The modulation of DAT function proceeds through rapid trafficking of the transporter to and from the plasma membrane. The mode of action of the A30P and A53T in such dual regulation of DAT activity differs from that of (-synuclein, and they also differ from one another in a highly prominent manner. In this proposal we will study in detail the mechanisms which underline such bimodal regulation of transporter activity, using cells co-transfected with alpha-synuclein and its subtypes, and the DAT cDNA, as well as in primary cultured neurons. The ability of the alpha-synuclein and its A30P and A53T mutants, and the identity of the structural components, which participate in direct protein:protein complex formation will be analyzed thoroughly in normal growth conditional states, through a battery of studies to include, immunology, transporter assays, immunocytochemistry and FRET. We will examine the role of oxidative stress in causing dysfunction of the three variants of alpha-synuclein. Studies will be conducted to determine if changes in alpha-synuclein/DAT interactions will reduce oxidative stress and cell death.-

Principal Investigator: SILVERMAN, RICHARD B
Grant Number: 1R01NS047331-01A1
Title: Celestrols for Treatment of Neurodegenerative Diseases

Abstract: The expression of molecular chaperones has been shown to suppress protein misfolding/aggregation and cellular toxicity phenotypes in model systems associated with Huntington's Disease, Alzheimer's Disease, Parkinson's Disease, and ALS. A feature common to diseases of protein conformation is the appearance of folded intermediates that self-associate to form protein aggregates and inclusions. The molecular chaperones Hsp90 and Hsp70 sequester damaged proteins that appear in cells exposed to physiological and environmental stress. The ability of molecular chaperones to suppress the cellular toxicities associated with expression of these "toxic" proteins may be due to the intrinsic properties of chaperones to capture and suppress the appearance of folded intermediates. Therefore, we propose that the identification of small molecules that elevate the expression of genes encoding heat shock proteins and molecular chaperones should lead to the development of novel therapies beneficial to the prevention of neurodegenerative diseases. The rationale for this proposal is based on results obtained by our laboratory and others who participated recently in a screening program organized by the NINDS, Huntington Disease Society of America, Hereditary Disease Foundation, and the ALSA to identify new drugs for treating these diseases. A search was carried out for drugs that activate the heat shock response; the most effective compound identified was the natural product celastrol. Synthetic analogs of celastrol will be prepared to optimize its effectiveness as a regulator of the heat shock response and a suppressor of neurotoxicity and to determine its mechanism of action as an activator of the heat shock response. To probe the function of celastrol as a potential therapy for neurodegenerative diseases, the following Specific Aims will be addressed: (1) Synthesize analogs of celastrol that induce the human heat shock response using a heat shock promoter-reporter assay in human tissue culture cells. (2) Determine the mechanism of action of celastrol (or an analog). The working model is that celastrol activates the heat shock response by inducing heat shock transcription factor HSF1. The mechanism by which HSF1 activity is induced by celastrol will be determined. It also will be determined whether celastrol, by virtue of its ability to activate the expression of chaperones, can reduce the aggregation and neurotoxicity of the Huntington Q64 protein expressed in a human SH-SY5Y neuroblastoma cell line. (3) Studies will be carried out to identify the binding target for celastrol using molecular biological and biochemical techniques. Identified target(s) will then be cloned and characterized. Results of these studies

Principal Investigator: SMEYNE, RICHARD J
Grant Number: 1R21NS045906-01A2
Title: Role of Environment in Neuroprotection

Abstract: PD is a debilitating neurological disorder that strikes 20 per 100,000 persons greater than 50 years of age. The cause of >90% of all PD cases are unknown. However, the discovery of the meperidine by-product 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has provided a useful model of Parkinsonism that appears to recapitulate the pathology of the disease seen in man. Exposure to this prototypical "environmental toxin" causes a selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). MPTP is a lipophillic molecule that rapidly enters the brain and is metabolized to MPP+ through a series of intermediates to MPP+ by the enzyme MAO-B. MPP + is a substrate for dopamine uptake mechanisms and it accumulates intraneuronally and interferes with complex I of the electron transport chain. We have recently shown that the glial cell is the critical cell for conferring protection or susceptibility to this toxin. Since PD is progressive, both in terms of cell loss and symptomotology, it would be of tremendous clinical value if there were cell biological, pharmacological or non-pharmacological methods that could attenuate cell loss; with or without interruption of the disease triggers. Alternatively, at the least, it would be important to slow the progression of cell loss once symptoms arose. There is a significant literature, dating back to the late 1700's that altering an animals' environment can lead to neurological changes. These changes are manifested as increased brain size, increased learning, and recently it has been shown that environment can increase neurogenesis. Recently, we have preliminary data to suggest that mice raised in an "Enriched Environment" (EE) are protected from MPTP toxicity. In this application, we will study and further establish the EE model. In addition, we will examine if the components (exercise, alterations in environmental complexity and/or social interactions) of the EE can confer neuroprotection. In addition, we will examine the role of the neurotrophin BDNF in EE-dependent neuroprotection. The work proposed and subsequent results generated in the application will be used as pilot data. We believe that the EE model may provide a new approach to prevention of PD symptomatology as well as other neurodegenerative disorders. -

Principal Investigator: SMEYNE, RICHARD J
Grant Number: 2R01NS039006-04A2
Title: Genetics of MPTP-Induced Parkinsonism

Abstract: Parkinson's disease (PD) is a debilitating neurological disorder that strikes 20 per 100,000 persons greater than 50 years of age. It is estimated that 1 million US citizens have PD, with adults over 60 having a 1 in 20 chance of getting PD. At an average per capita cost of $6000.00 year/patient, the total cost of the disease approximates $6 billion dollars, of which 85% is borne to private and government insurance agencies. Since the population of the world is getting progressively older, the number of people suffering from this disease should substantially increase within the next several decades. The cause of >90% of all PD cases is unknown. Current hypotheses on the etiology of idiopathic PD (IPD) state that there is an interaction of some as yet unknown environmental agent with a genetic predisposition to its effects. The discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has provided a useful model of Parkinsonism that appears to recapitulate the pathology of the disease seen in man. Exposure to this prototypical "environmental toxin" causes a selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). In mice, the effects of MPTP are strain dependent. We have used a QTL analysis to demonstrate that the gene underlying strain differences is located on chromosome 1. Within this chromosomal region, one gene: glutathione-S-transferase pi2 functions within the detoxification pathway for exogenous agents. In this application, we propose to study the structure and function of this gene and its related family members. Four specific aims are proposed: 1) Determine if there are any differences in the sequence and expression of GSTp2 and related family members in MPTP-resistant and sensitive strains of mice. 2) Examine the effects of blockade or transfer of GSTpi on cell death following administration of MPTP in vitro and in vivo; 3) Develop the rotenone model of experimental Parkinsonism in mice and determine if GSTp2 is altered in response to rotenone; 4) Determine if there are structural or expression differences in GSTpi levels in humans with Parkinson's disease. The results of this study should lead to a better understanding of the pathogenesis of experimental and possible human Parkinson's disease. This identification of GSTp2 as a candidate gene could also lead to the identification of diagnostic measures and point to potential therapies for early intervention in this devastating illness. -

Principal Investigator: SMITH, AMANDA D
Grant Number: 1K01NS045698-01A1
Title: Endogenous neuroprotective agents in Parkinson's disease

Abstract: The present application describes the research and career plan laid out for my development into an independent, productive, and well funded investigator in the area of the neurobiology of neurodegenerative disease. The research plan that is proposed investigates the role of circulating insulin like growth factor (IGF-1) and associated proteins in protection of the nigrostriatal dopamine (DA) pathway against oxidative stress induced by 6-hydroxydopamine (6-OHDA) and the nature of this protection. The loss of DA neurons in this pathway underlies the motor dysfunctions observed in patients with Parkinson's disease (PD). Forced use of the impaired forelimb for 7 days in a unilateral 6-OHDA lesion model of Parkinson's disease, ameliorates behavioral asymmetry and restores DA content in the striatum when commenced immediately after or prior to neurotoxic insult. The mechanism by which forced use protects against 6-OHDA toxicity is unknown. Moreover, whether forced use protects the nigrostriatal pathway from degenerating, rescue cells in danger of degenerating in the absence of intervention, or promotes sprouting, is not known. Physical exercise by treadmill or running wheel has been shown to increase the brain uptake of IGF-1 from the circulation and this IGF-1 has been shown to mediate exercise-induced increases in neurogenesis and brain derived neurotrophic factor mRNA in the hippocampus. Thus, it may be surmised that forced use protection is mediated via increases in brain IGF-1 subsequent to increases in circulating IGF-1. Our preliminary data using Fluoro-jade B as a marker of degeneration suggests that forced limb use prevents the nigrostriatal pathway from degenerating. Further, a preliminary screen of altered genes after 6-OHDA and 6-OHDA +/- forced limb use, with microarray analysis suggests that IGF-1 may be involved. In the present proposal, we will: 1) Further examine the impact of forced use/disuse on the anatomical and functional state of DA neurons using behavior, biochemistry and histological analyses; 2) investigate the role of IGF-1 in forced limb use-induced protection, whether this effect can be mimicked by systemic administration of IGF-1 and whether subsequent up-regulation of other trophic factor signaling (i.e. GDNF and BDNF) is involved; and 3) examine whether the protective effects of forced limb use and IGF-1 are mediated via activation of the pro-survival phosphatidylinositol 3-kinase (PI 3K)/Akt and extracellular signal-regulated kinase (ERK) signaling cascades. The career development plan in the present proposal focuses on providing me with the technical skills needed to accomplish the Aims outlined in the present proposal. Further, it will provide the skills and

Principal Investigator: SNYDER, HEATHER M
Grant Number: 5F31NS042510-02
Title: a-Synuclein Linkage to the Proteasome

Abstract: Unavailable

Principal Investigator: Sonsalla, Patricia K
Grant Number: 5R01NS041545-04
Title: Dopamine Homeostasis, Vesicles & Neurodegeneration

Abstract: Parkinson's disease is a debilitating motor impairment disorder due to loss of nigral dopamine neurons. Mitochondrial defects in PD patients implicate energy impairment and metabolic stress as potential factors in its etiology. Moreover, DA oxidation products may add to the oxidative burden within DA neurons which, coupled with a persistent metabolic stress, may lead to neurodegeneration. Epidemiological studies link PD with environmental exposure to substances such as pesticides. - Many pesticides are mitochondrial inhibitors. A potential form of protection against mitochondrial toxins (i.e., MPP+) may be their sequestration into synaptic vesicles of DA neurons. The goal of this project is to gain an understanding of the role of vesicles, the vesicular monoamine transporter (VMAT2) and DA in modulating neurodegeneration produced by mitochondrial toxins. One hypothesis is that the actions of mitochondrial toxins can be modulated in contrasting ways depending on whether the toxins are sequestered into vesicles. If sequestered, toxin exposure could be abrogated. In contrast, disruption of vesicular function toxin could lead to disturbed DA homeostasis and enhanced toxicity since it would remove the toxin from interaction with mitochondria. In Aim 1 several mitochondrial toxins will be examined for their ability to interfere with vesicle function (i.e. to inhibit DA uptake into isolated rat membrane vesicles). In aim 2, rat mesencephalic cultures or rat striata will be exposed to mitochondrial toxins following VMAT2 inhibition to determine if toxicity is modified. To examine the hypothesis that disturbed DA homeostasis contributes to degeneration produced by metabolic stress, two approaches will be used. First, DA will be depleted prior to exposure of culture or rat striata to a mitochondrial inhibitor. Second, vesicle contents (DA) will be released into the cytosol after exposure to the mitochondrial toxin to examine if augmented disruption of DA homeostasis during the metabolic stress enhances toxicity. Additionally, the hypothesis that substances that are not themselves mitochondrial inhibitors, but can disrupt DA storage in vesicles may amplify damage during episodes of metabolic stress will be examined in Aim 3. In aim 4 the hypothesis that early events such as oxidative stress leads to loss of vesicle function, disruption of DA homeostasis and exacerbation of neurodegeneration produced by toxins will be investigated. Isolated vesicles will be tested for their sensitivity to oxidizing and reducing conditions. Results from these studies will provide novel and relevant information as to the contribution of VMAT2 containing vesicles in neuroprotection as well as in neurodegeneration of DA neurons during metabolic

Principal Investigator: SORTWELL, CARYL E
Grant Number: 1R03NS048188-01
Title: Evaluation of Hypoxia in Grafted Dopamine Neurons

Abstract: Intracerebral neural grafting strategies for neurological disorders are limited by the poor survival rate of grafted cells. For example, the survival rate of dopamine (DA) neurons grafted in parkinsonian animal models and in clinical trials with Parkinson s patients is merely 5-20%. Critical to clinical success is the development of methods whereby grafted DA neuron viability and reinnervation of the host striatum are markedly increased. The focus of this R03 application is to directly examine the role of hypoxia in intracerebral grafts utilizing a well-established paradigm, grafts of mesencephalic DA neurons. Our research indicates that massive apoptosis of grafted mesencephalic cells occurs within the first few days after transplantation and then sharply diminishes. This time course of grafted DA neuron death closely parallels the delay in host vascularization of the grafted cells. The lack of blood-borne oxygen, or hypoxia, experienced by the grafted cells during the immediate post-grafting interval is a likely candidate to trigger apoptotic cell death. However, the role of hypoxia in limiting graft survival has never been directly assessed. The overall hypothesis of this proposal is that grafted DA neuron survival is severely limited by hypoxia during the early post-transplantation interval when grafted cells are not adequately vascularized. Identification of hypoxia as a significant constraint on graft survival would direct future strategies aimed at enhancing intracerebral grafts of numerous cell types (primary cells, stem cells) implanted to treat a wide range of neurological disorders.-

Principal Investigator: STANDAERT, DAVID G
Grant Number: 5R01NS034361-09
Title: NMDA RECEPTORS--REGULATION OF BASAL GANGLIA FUNCTION

Abstract: Glutamate is the principal excitatory neurotransmitter in the brain and has an important role in the regulation of movement. N-methyl-D-aspartate (NMDA) glutamate receptors are of particular interest because they are involved in long-term processes such as neural adaptation and memory. Drugs acting at NMDA receptors have important therapeutic potential in human Parkinson's disease. In particular, recent work has suggested that changes in NMDA receptor properties may be responsible for the development of the motor complications of levodopa therapy, such as wearing off and dyskinesias. Such motor complications occur in the majority of patients with Parkinson's disease and are frequently the principal cause of disability. NMDA receptors are assembled from proteins from two gene families, and receptors with different composition have distinct properties. The functions of the receptors are further regulated by differential trafficking and phosphorylation. Our investigations have revealed that neurons which serve different functions in the circuitry of the basal ganglia express different types of NMDA receptor subunits. In models of Parkinsonism, striatal NMDA receptors are modified. The most significant changes are not alterations in the level of gene expression, but rather are changes in the assembly, phosphorylation, and synaptic localization of the protein subunits. In this project, we will employ a variety of techniques to establish the nature and mechanisms of the modifications of basal ganglia NMDA receptors produced by chronic dopamine depletion and dopamine replacement therapy. The long term goal of this work is to gain insight into the cause of wearing off and dyskinesias in Parkinson's disease, and develop better treatments for this common and disabling neurological disorder. -

Principal Investigator: STAROPOLI, JOHN F
Grant Number: 1F31NS048668-01
Title: Parkin and Its Regulation of Neuronal Apoptosis

Abstract: Mutations in parkin underlie an autosomal recessive form of Parkinson's disease, the second most common neurodegenerative disease. To test a working model of parkin as a component of a multi-subunit, SCF-like ubiquitin ligase complex that protects dopamine neurons from apoptosis, other components of the complex, including sel-10 and cullin-1, will be dowregulated by RNA interference in murine primary neuronal cultures. Downregulation of these components will be evaluated for potentiation of dopamine neuron apoptosis and compared to the effects of downregulating parkin itself. To test the hypothesis that a candidate substrate of the parkin-associated complex, cyclin E, is a key mediator of the apoptotic cascade(s) against which wildtype parkin normally protects neurons, pharmacological inhibition of cyclin E-associated activity will be evaluated for rescue of dopamine neurons in the context of parkin, sel-10, or cullin-1 downregulation. Finally, lentivirus-mediated overexpression of parkin in the same primary culture system will be assessed for protection of dopamine neurons from neurotoxins as compared to overexpression of mutant forms of parkin, including clinically defined mutations and forms deleted in the ubiquitin homology and RING domains.-

Principal Investigator: STARR, PHILIP A
Grant Number: 2K08NS002201-04A1
Title: Pallidal Physiology in Human and Primate Dystonia

Abstract: (provided by applicant: Dystonia is a movement disorder defined as a syndrome of sustained muscle contractions, causing twisting and repetitive movements, and abnormal postures. It is often devastating and its pathophysiology is poorly understood. Recently, attempts have been made to understand movement disorders in terms of alterations in a loop circuit involving the cortex, basal ganglia and thalamus. The globus pallidus internus (GPi) occupies a critical position in this circuit since it is the major output structure of the basal ganglia. Another movement disorder, Parkinson's disease (PD), has been found to be associated with excessive and abnormally patterned GPi activity. This finding has led to improved surgical treatments for PD by pallidal inactivation. In contrast to PD, a better understanding of dystonia has been hampered by a lack of data on the physiology of the basal ganglia in this condition, and by the lack of a well-characterized nonhuman primate model of dystonia. Both problems are addressed in this ongoing study. In the initial three years, we recorded and analyzed 283 pallidal units in 14 patients with dystonia, 74 units in a normal Rhesus macaque, and 75 units from four patients with Parkinson's disease. Human patients undergo electrophysiologic mapping as a routine part of pallidal surgery for movement disorders. We showed that, in comparison with normal macaque, dystonia is associated with reduced neuronal activity in the GPi in most but not all cases, increased bursting activity in GPi, and a slight reduction in activity in the external pallidum. These data lend support to a model of dystonia in which both direct and indirect pathways of the basal ganglia are overactive. However, some cases show little abnormality in discharge rate or pattern, motivating a continued search for a "signature" abnormality in dystonia. In addition, we began development of a model of focal arm dystonia in the Rhesus macaque, in which dystonia is generated by repetitive performance of a skilled motor task. In the proposed continuation, spontaneous and movement-related discharge in GPi will be studied in ten additional dystonia patients, with a new emphasis on neuronal responses to sensory feedback and cross correlation of simultaneously recorded cells. In the macaque model of dystonia, the effect on motor performance of lesioning the globus pallidus will be analyzed. The experiments test the following hypotheses: 1) Idiopathic dystonia in humans is associated with abnormal neuronal synchrony and abnormal responses to somatosensory examination in the GPi. 2) In non-human primates, dystonia induced by a repetitive arm movement task can be ameliorated by lesions of the GPi, establishing the relevance of this

Principal Investigator: SUBRAMANIAN,
Grant Number: 7R01NS042402-04
Title: Intranigral Transplantation in Parkinsonian Monkeys

Abstract: Recent investigations indicate that dopaminergic (DArgic) neurons in the substantia nigra (SN) secrete dopamine not only in their axonal terminals within the striatum but also via their dendrites within the SN pars reticulata (SNr) and that loss of dopamine in the SNr may have a role in the development of parkinsonism in primates. As a corollary, restoration of both nigral and striatal dopamine inputs may produce better recovery of function in Parkinson's disease than restoration of dopamine inputs in the striatum alone. Therefore, the PI proposes to examine the effects of combined DArgic fetal ventral mesencephalic (FVM) cell transplantation into the SN and the striatum in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-treated hemiparkinsonian (HP) monkeys and compare the results with FVM transplants in the striatum or SN alone. Animals will be periodically assessed by investigators blinded to the type of transplantation using a behavioral battery of tests (BBT). All animals will be treated with intracarotid MPTP injections to cause a stable HP state and briefly treated with oral levodopa to verify responsiveness to DArgic therapy prior to randomization into 4 equal groups (1-4). Microelectrode recordings of neuronal activity and magnetic resonance imaging (MRI) will be used to guide all transplantation procedures. In specific aim 1 (SA 1), group 1 animals will receive simultaneous FVM transplants into both striatum and the SN, group 2 animals will receive striatal FVM transplants, group 3 animals will receive FVM transplants into the SN and group 4 animals will receive "control" fetal tissue transplants into the SN. Periodic BBT assessments and immunochemical assessment of the transplanted animals compared across groups 1-4 will be used to test the hypothesis that combined striatal and nigral FVM transplants ameliorates parkinsonism to a greater extent than striatal FVM or nigral FVM transplants alone. In SA 2, neuronal recordings will be obtained before and after tissue transplantation from all 4 groups of animals from the SNr and the subthalamic nucleus (STN) and compared. This experiment will examine the hypothesis that striatal FVM transplantation will alter neuronal discharge patterns in both SNr and in the STN, while nigral FVM transplantation will alter neuronal discharge patterns in the SNr only. In SA 3, dopamine levels will be measured in vivo using microdialysis before and after nigral FVM transplantation from the SN and STN in group 3 and group 4 animals. This experiment will test the hypothesis that nigral FVM transplants restore dopamine content in the SN but do not effect dopamine content in the STN. These 3 experiments will objectively evaluate the role of restoring DArgic

Principal Investigator: SUN, GRACE Y
Grant Number: 1R13NS047414-01
Title: Conference on Oxidative Mechanisms in Neurodegeneration

Abstract: This application seeks funds for partial support of US investigators to attend a symposium entitled "Oxidative mechanisms in Neurodegenerative disorders" to be held in Guilin, China, August 9-13, 2003. The symposium is a satellite to the International Society of Neurochemistry/Asian Pacific Society of Neurochemistry (ISN/APSN) that will be held in Hong Kong, August 2-7. The Central Nervous System (CNS) is highly susceptible to oxidative stress, which alters many metabolic pathways leading to cellular dysfunction. Since increase in oxidative stress has been implicated in the pathophysiology of a number of age related neurodegenerative disorders, including AIzheimer's disease, Parkinson's disease and stroke, this symposium brings together world-class scientists to share new findings and insights on oxidative mechanisms underlying these neurodegenerative disorders. In addition to sessions focused on oxidative mechanisms associated with Alzheimer's disease, Parkinson disease, stroke, and receptor signaling pathways, a special session will be dedicated to discussing novel methods for invervention and prevention. Thus, this symposium not only provides mechanisms for international scientists and young investigators to focus on an import topic of immense interest to neuroscientists, it also provides opportunities for investigators outside of hina to learn and interact with Chinese investigators in basic and clinical aspects of neurodegenerative diseases. In addition, plans are in progress to provide rapid publication of the symposium proceedings in an internationally recognized neuroscience journal. -

Principal Investigator: SURMEIER, DALTON
Grant Number: 5R37NS034696-10
Title: DOPAMINERGIC AND MUSCARINIC SIGNALING IN THE STRIATUM

Abstract: Parkinson's disease (PD) is a disabling neurodegenerative disorder that is expected to affect as many as 1,000,000 Americans this year. Human and animals studies have shown that parkinsonism results from the degeneration of nigrostriatal dopaminergic neurons. Currently, treatment strategies for PD patients are limited. Gaining a better understanding of how striatal function is altered by the disease should broaden the range and efficacy of treatments. In the last funding period we focused on how dopamine and acetylcholine modulate the properties of voltage dependent ion channels in identified normosensitive striatal neurons. These studies have provided fundamental new insights into how these neuromodulators control the excitability of striatal neurons. Now, we are in a position to take the next step toward understanding the pathophysiology of PD - namely, how does the depletion of intrastriatal dopamine alter striatal function? Simply stated, our central goals are 1) to determine how DA depletion alters the regulation of voltage-dependent ion channels in striatal medium spiny neurons and 2) to determine how this adaptation alters their integrative, state-dependent behavior. To this end, we will determine how DA depletion alters Na+ and Ca2+ currents and their modulation by D2 (Specific Aim 1) and D1 receptor activation (Specific Aim 2) in identified striatal neurons. These experiments will rely upon voltage-clamp and single cell reverse transcription-polymerase chain reaction (scRT-PCR) approaches in acutely isolated striatal neurons - techniques with which we have an established track record. The proposed studies will employ newly developed mouse transgenic models in which striatal dopamine levels are profoundly reduced, mimicking the state found in advanced PD. Adaptations in the signal transduction pathways linking receptors to channels will be characterized using a combination of pharmacological, molecular and transgenic strategies. Inferences drawn from this work about adaptations in the mechanisms governing state transitions and repetitive spike activity will be explicitly tested using current- and voltage-clamp techniques in a novel corticostriatal slice preparation where medium spiny neurons exhibit state-dependent behavior resembling that seen in vivo (Specific Aim 3). -

Principal Investigator: TEPLOW, DAVID B.
Grant Number: 5R01NS044147-02
Title: Formation and Function of Prefibrillar ABeta Assemblies

Abstract: We hypothesize that amyloid Beta-protein (Abeta) assembly is a seminal neuropathogenetic process in Alzheimer's disease (AD) and in cerebral amyloid angiopathy (CAA). If so, controlling Abeta assembly could be of therapeutic value. Over the last decade, we have worked to elucidate the pathways of Abeta self-association in vitro, to identify assembly intermediates, and to evaluate the neurotoxic activities of these structures. This work led to the discovery of the amyloid protofibril, later found to be neurotoxic in vitro and in vivo, and to be linked to an "Arctic" form of AD. Recently, novel chemical cross-linking studies have revealed the existence of smaller oligomeric structures (paranuclei), which are formed rapidly by Abeta (1-42) but not by Abeta (1-40). The strong association of Abeta (1-42) with AD thus may result from Abeta (1-42)-specific assembly events occurring at the earliest stage of self-association, oligomerization. We also have described a novel helix-rich oligomeric assembly intermediate. We predicted that an Asp23->Asn amino acid replacement would affect the rate of formation of this intermediate and of fibrils. Interestingly, the importance of this site has been proven in humans through the discovery of an Iowa kindred suffering from an early onset form of CAA caused by this exact substitution. In this proposal, we will examine the thermodynamics and structural biology of early Abeta assembly reactions in order to understand the fundamental factors controlling these reactions and to characterize the structures formed. In concurrent experiments, we will examine the neurotoxic activities of the Abeta assemblies to establish which may be of most relevance pathobiologically. Our studies will provide a better understanding of the mechanisms of formation and the biological activities of Abeta assemblies and of general principles of amyloid formation and protein misfolding. Our experimental plan comprises four specific aims. Aim 1. To elucidate the thermodynamics of early Abeta assemblies and Abeta fibril formation. Aim 2. To determine the structural features of early Abeta assemblies. Aim 3. To determine the structure and mechanism of action of oligomeric Abeta fibrillogenesis inhibitors. Aim 4. To determine the biological activity of early intermediates. -

Principal Investigator: TESTA, CLAUDIA M
Grant Number: 5K08NS044267-03
Title: Mitochondrial dysfunction in neurodegenerative disease

Abstract: Like most neurodegenerative disorders, Parkinson disease (PD) has a chronic, slowly progressive course, selective neuronal loss, and a small percentage of familial cases caused by mutations in widely expressed genes. A simplified, reproducible and relevant model system that allows study of progressive neuronal injury would permit us to examine mechanisms of chronic neurodegeneration in PD, and allow us to screen potential neuroprotective agents. Organotypic "slice" culture models offer major advantages in that they are simplified compared to in vivo models, yet unlike dissociated cell cultures they involve the use of mature neurons, remain viable in culture for months, and maintain substantial intact circuitry and neuronal-glial interactions. We propose to characterize and use such a model to specifically examine mechanisms of neuronal injury in PD. Mitochondrial dysfunction has been proposed as a factor underlying dopaminergic cell loss in PD. There is growing evidence of decreased mitochondrial function and increased oxidative stress in human PD. In a new animal model of PD, systemic infusion of the mitochondrial toxin rotenone, an organic pesticide, causes degeneration of the nigrostriatal pathway that is highly selective, even in the presence of global mitochondrial inhibition. In the current proposal we will: 1) Optimize and characterize a rotenone model of PD in chronic organotypic slice cultures. We present data from preliminary studies demonstrating the successful use of slices containing substantia nigra pars compacta dopaminergic neurons for this purpose. 2) Exploit the unique advantages of this system to investigate the mechanisms of action of mitochondrial inhibition. We will examine the role dopamine itself plays in neuronal vulnerability, and look for evidence of oxidative damage and apoptotic cell death. 3) Investigate the interaction of genetic defects with environmental stressors in PD. We will use transgenic mouse models to examine how rotenone interacts with genetic mutations that produce familial PD. We will study how underlying genetic lesions that affect oxidative stress and apoptosis pathways may predispose cells to damage from exogenous toxins. 4) Test potential neuroprotective agents in a model of chronic neurodegeneration that is highly relevant to PD. The research outlined above is part of a customized five-year plan of training and career development for the Principal Investigator. The proposal includes active mentoring by experienced scientists, access to diverse resources, and an environment uniquely suited to help the PI develop as an independent physician scientist.-

Principal Investigator: TRAYNELIS, STEPHEN F
Grant Number: 3R01NS036654-06S1
Title: Control of glutamate receptor activation

Abstract: Unavailable

Principal Investigator: Traynelis, Stephen F.
Grant Number: 5R01NS036654-07
Title: Control of glutamate receptor activation

Abstract: N-methyl-D-aspartate-selective glutamate receptors (NMDA-Rs) mediate a slow component of excitatory synaptic transmission in the CNS and are involved in synaptic plasticity, learning, and memory. Activation of NMDA-Rs can contribute to the initiation and maintenance of seizures. In addition, stimulation of NMDA-Rs by extracellular glutamate that accumulates during ischemia can lead to cytotoxic levels of Ca2+ and neuronal death. Given this potential danger of NMDA-R overactivation, it is not surprising that NMDA-R function is tightly regulated by a number of endogenous extracellular ions including protons. Extracellular protons inhibit NMDA-R function completely by binding to a single binding site (Hill slope about 1) with a pKa of 7.0 (125 nM H+) or 7.4 (50 nM H+) for recombinant NRI/NR2A and NRI/NR2B receptors. Despite the potential importance of NMDA-R function, an understanding of the basic mechanisms by which NMDA-R channels open is lacking. No conceptual model exists for NMDA receptor function that explains both single channel and macroscopic receptor properties. The experiments outlined for the next period exploit our recent success obtaining excised outside-out patches that contain only one active NMDA-R channel. This single channel approach will be combined with macroscopic current recording and quantitative modeling to explore the mechanism of NMDA-R activation (spec. aims 1-3). Detailed functional information about NMDA-R gating will be required to maximize interpretation of structural information. Understanding NMDA-R gating is also a pre-requisite to understanding both the proton sensitivity of gating and the function of the therapeutically interesting compounds that regulate proton inhibition (aims 4-5). Five questions will be addressed. I. Is NMDA receptor function controlled by two independent gates? 2. Can single channel kinetics and macroscopic current response time course be reconciled by multi-gate models? 3. Do the glycine and glutamate binding subunits contribute kinetically distinct gates to the NMDA receptor pore? 4. Do protons and phenylethanolamines reduce the probability that an agonist-bound receptor will open? 5. Is the structural basis for H+ sensitivity of NMDA and G1uR6 receptors contained in the transmembrane linker regions? Together, these experiments will help define a unifying theory for NMDA receptor function that accounts for single channel and macroscopic behavior. In addition, evaluation of the hypothesis that protonation of a few key residues inhibits channel opening without changing other features of receptor function will increase our understanding of the structural nature of how glutamate receptors open and close in response to full and

Principal Investigator: Traynelis, Stephen F.
Grant Number: 3R01NS036654-07S1
Title: Control of glutamate receptor activation

Abstract: Unavailable

Principal Investigator: TROY, CAROL M
Grant Number: 5R01NS043089-04
Title: Downstream Regulators B-amyloid Induced Neuronal Death

Abstract: Our overall aim is to determine the molecular mechanisms of beta-amyloid-induced neuronal death. Deposition of insoluble Abeta, together with tangle formation and neuronal loss, are hallmarks of Alzheimer's disease. Recent studies show that increased Abeta induces synaptotoxicity, a parameter which correlates with cognitive decline in AD. Evidence from our work and from other laboratories shows that insoluble Abeta induces apoptosis in cultured neurons. The Abeta-mediated death pathway has many similarities to the trophic factor deprivation (TFD) mediated death pathway. These include induction of c-fos and c-jun, use of cell cycle components, activation of the JNK cascade, and protection by bcl-2. We have clearly demonstrated that caspase-2 is necessary for Abeta as well as for TFD mediated death and that although there is parallel activation of caspase-3 it is not sufficient to induce death. However, data obtained from caspase-2 null mice indicate that the death pathways of these two stimuli are not identical: cultured sympathetic neurons from these mice are protected from Abeta but not from TFD. We propose the hypothesis that Abeta and TFD both execute death via a caspase-2 mediated pathway but that the relative expression of caspase regulators (IAPs and Diablo/SMAC) determines different alternative pathways for Abeta and TFD. We additionally propose the hypothesis that JNK activation leads to induction of Fas and recruitment of RAIDD activating caspase-2. By contrasting and comparing the mechanisms employed by these two death stimuli we can further dissect the Abeta-mediated death pathway. We will examine these hypotheses with the following specific aims: 1. To determine which caspases are activated (processed) during Abeta and TFD mediated death and which caspases can execute Abeta and TFD induced death. 2. To determine whether there is differential expression and regulation of MIAP3 or Diablo after Abeta or TFD. 3. To determine whether activation of the JNK cascade leads to caspase-2 activation. 4. To determine whether the molecular components of the Abeta death pathway, identified in the preceding Aims, are altered in a mouse model of AD and/or in human AD brain. -

Principal Investigator: TROYER, MATTHEW D
Grant Number: 5K08NS002251-05
Title: OXIDATIVE STRESS/ALPHA-SYNUCLEIN IN PARKINSON'S DISEASE

Abstract: Parkinson's disease (PD), the second most common neurodegenerative disorder, results from selective loss of midbrain dopamine neurons. Both oxidative stress and intracellular aggregation of proteins, including the protein alpha-synuclein, are implicated in this degeneration. However, the source of oxidative stress, the mechanism of alpha-synuclein deposition, and the relationship between them are unknown. We will examine the role of the neurotransmitter dopamine in generating oxidative stress by manipulating its synthesis, degradation and vesicular transport and measuring production of reactive oxygen species. We will also investigate the effect of dopamine-mediated oxidative stress on alpha-synuclein deposition in model cell culture systems. We will then use this information to determine conditions that promote alpha-synuclein deposition in transgenic mice expressing wild type human alpha-synuclein or a mutant alpha-synuclein that causes an autosomal dominant form of PD. Thus we hope to identify factors that promote oxidative stress in dopamine neurons and to better understand the mechanisms and significance of alpha-synuclein deposition in PD. This work will be conducted under the sponsorship Dr. Robert Edwards in the Departments of Neurology and Physiology at UCSF. Through this work I will learn new techniques in molecular biology, biochemistry and imaging that I will continue to apply to PD. Dr. Edwards and I have developed a training program that includes laboratory research, coursework and didactics that will enable me over the next five years to become an independent investigator concentrating on the neurobiology of PD. In the long term I intend to spend 75% or more of my time dedicated to scientific investigation of PD, and to direct my clinical activities toward patients with PD and other movement disorders. -

Principal Investigator: VAN DER WALT, JOELLE
Grant Number: 1L30NS050033-01
Title: Mitochondrial dysfunction in Parkinson's disease

Abstract: Unavailable

Principal Investigator: VEGA, QUINN C
Grant Number: 1R15NS048043-01
Title: Analysis of RET and GFRa-1 down regulation

Abstract: The GDNF/RET/GFRa-1 comlex is involved in many physiological processes including neural migration and neuronal survival. GDNF has also been shown to ameliorate the affects of Parkinson's disease in mice, primates and, most recently, humans. Although RET signaling has been studied in some detail, the mechanism by which this signal pathway is down-regulated after ligand binding is less well understood. Down regulation is critical since it is known that RET, when activated constitutively, leads to unregulated cell growth. It will also be critical to understand, with respect to Parkinson's disease patients, how long term treatment with GDNF affects the signaling pathway and its component parts. The focus of this work will be to monitor RET and the co-receptor prior to and after GDNF treatment with respect to down regulation. Given the importance of these proteins in development, disease and the potential treatment of disease, it will be critical to determine not only how these proteins are activated but what happens after the proteins have been activated. For this project, two potential mechanisms of down-regulation will be measured. With the assistance of undergraduate and master's students, wild type and mutant RET and GFRa-1 proteins will be monitored for changes in transcription, internalization or degradation. Transcriptional regulation will be measured using northern blots and rt-PCR analysis. With respect to internalization, RE'I" and its co-receptor will be monitored using receptor labeling, internalization measurements and co-localization using confocal microscopy of both wild type and mutant proteins. Similar processes will be used to monitor degradation. Finally, once the down-regulation mechanisms have been established, the potential role of disrupted down-regulation in disease progression will be analyzed.-

Principal Investigator: WALKER, PAUL D
Grant Number: 5R01NS039013-04
Title: SEROTONIN CONTROL MECHANISMS OF BASAL GANGLIA FUNCTION

Abstract: Attempts to develop new and effective treatments for movement disorders such as Parkinson's disease have been hampered by an insufficient knowledge of how basal ganglia receptor systems adapt to the consequences of dopamine depletion. This research focuses on determining the role of upregulated serotonin 2A receptors, which we hypothesize provide a mechanism for serotonin to exert greater control over basal ganglia transmission and locomotor function under conditions of dopamine depletion. Our preliminary studies indicate that the target of the serotonin 2A receptor mechanism is the DIRECT striatonigral pathway which utilizes tachykinin neuropeptides colocalized with GABA. New experiments of this application will test the central hypothesis that: upregulated serotonin 2A receptor signaling provides a mechanism for serotonin to enhance striatonigral transmission under conditions of dopamine depletion which influences basal ganglia function and animal behavior. In Specific Aim 1, we will determine the functional consequences of an upregulated serotonin 2A receptor system on serotonin signal transduction within the dopamine depleted striatum by measuring serotonin 2A receptor binding, its linkage to phosphoinositol hydrolysis, its modulation of striatal membrane excitability, and its ability to trans-synaptically regulate striatal tachykinin and GABA expression. In Specific Aim 2, we will determine if tachykinin striatonigral neurons react to the stimulation of upregulated serotonin 2A receptors in the dopamine depleted animal by increasing tachykinin and GABA transmission in the substantia nigra. We will also study the impact of this regulation on locomotor behavior. Finally, in Specific Aim 3, we will determine how an upregulated serotonin 2A receptor system influences the ability of the striatonigral system to regulate basal ganglia dopamine and GABA metabolism, and how these systems influence behavioral recovery of the dopamine depleted animal. Information obtained from these studies will contribute to a better understanding of basal ganglia function and may change how serotonin pathways are considered when designing new pharmacological strategies for diseases which affect dopamine transmission. -

Principal Investigator: WALLACE, DOUGLAS C
Grant Number: 5R01NS041850-05
Title: ANT Defects in Neurodegenerative Diseases

Abstract: We propose to investigate the role that mutations in the mitochondrial adenine nucleotide translocator (ANT) genes play in neuromuscular degenerative diseases. The ANTs energetically link the mitochondria with the cytosol by exchanging mitochondrial ATP for cytosolic ADP. They are also thought to be an integral component of the mitochondrial permeability transition pore (mtPTP) and, therefore, central to apoptosis. Humans have three ANT isoform genes: the heart-muscle ANT1, the inducible ANT2, and the constitutive ANT3. Mouse has two ANT isoforms: Antl expressed in the skeletal muscle, heart, and brain and analogous to human ANT1, and Ant2 expressed in all tissue but skeletal muscle and analogous to human ANT2 plus ANT3. Mutations in human ANT1 have recently been shown to cause autosomal dominant progressive external opthalmoplegia (adPEO), a neuromuscular disease associated with multiple mitochondrial deletions. Previously, we generated an Antl knockout mouse which has similar features to adPEO and thus is an excellent model for ANT1 disease. Recently, we have also prepared a LoxP-Ant2-LoxP mouse permitting the tissue-specific elimination of Ant2. To further define the role of ANT defects in neuromuscular diseases, we propose four Specific Aims. Specific Aim 1 is to investigate the pathophysiology of ANT1 disease. We will determine the neuromuscular phenotype, physiological defects and Mitochip gene expression profiles of Ant1 +/- and -/- mice. Specific Aim 2 is to explore the nature of ANT2 and ANT3 disease. We will combine our LoxP-Ant2-LoxP mice with different Cre-promoter constructs to generate females that are Ant2 heterozygotes and animals that are Ant2-deficient in heart, cortex and cerebellum. These animals will be characterized for their phenotype, physiology and Mitochip gene expression profiles. We will also generate mice that are Ant1 and Ant2 null mutants in their livers and use the liver mitochondria to investigate the role of the ANTs in the mtPTP and in apoptosis. Specific Aim 3 takes advantage of the phenotypes exhibited by our Ant1 and Ant2 mouse mutants as guides to identify neuromuscular disease patients likely to harbor ANT defects. We will then use our isoform-specific antibodies and Mitochip expression profiles to screen for ANT defects and identify the mutant genes by ANT gene sequencing. Finally, in Specific Aim 4, we will use the Ant1 -/- mouse as a model system to develop an AAV gene therapy procedure for ANT1adPEO. These studies, upon completion, will provide critical new insights into the role of ANTs in mitochondrial function and neurodegenerative diseases. -

Principal Investigator: WEST, ANTHONY R
Grant Number: 1R01NS047452-01A1
Title: Characterization of Striatal Nitric Oxide Signaling

Abstract: Recent studies have shown that striatal nitric oxide (NO)-producing interneurons play an important role in modulating striatal neural activity and motor behavior. NO is a gaseous neurotransmitter produced by NO synthase (NOS) following glutamate receptor activation. NO diffuses freely through biological membranes and stimulates guanylyl cyclase (GC) and dopamine (DA) release processes critically involved in the generation of motor activity. Studies have shown that striatal NO interneurons receive inputs from the cortex and substantia nigra. However, the influence of these afferents on NOS activity remains to be determined. Additionally, the impact of NO-GC signaling pathways on the synaptic activity of medium spiny neurons (MSNs) is poorly understood. Therefore, the proposed studies plan to examine the afferent systems involved in activating striatal NOS and determine the impact of NO-GC signaling on MSN membrane activity using both in vivo and in vitro preparations. Aim 1 will utilize electrochemical microsensor measures of extracellular NO levels to determine the role of DA receptors in modulating the glutamatergic activation of striatal NOS. Aim 2 will use in vivo intracellular recording techniques in conjunction with microdialysis to determine the influence of NO signaling cascades on the bistable membrane activity of MSNs. Parallel studies will be performed in brain slice preparations to determine the role of GC signaling pathways in mediating the influence of NO on synaptic activity. We hypothesize that activation of corticostriatal afferents will augment striatal NO production in a manner that is differentially modulated by ongoing D1 and D2 DA receptor activation; moreover, activation of NO signaling will increase the excitability of MSNs via a GC-dependent mechanism, in a manner that is potentiated by D1 receptor activation. We believe that these studies will shed light on the mechanisms involved in the integration of dopaminergic and corticostriatal signaling by striatal neurons and suggest novel treatment strategies for Parkinson's disease. -

Principal Investigator: WIEDAU-PAZOS,
Grant Number: 1K08NS002240-01A2
Title: Gsk-3beta & beta-Catenin in pathophysiology of FTDP-17

Abstract: This proposal will enable the applicant to become an independent researcher in the field of inherited neurodegenerative disorders. It builds upon the candidate's background in aging research and implements a comprehensive career development plan that aims to 1) expand the breadth of research skills in the area of cell biology and genetics and enhance current research skills, 2) fill knowledge gaps in the understanding of cellular pathways in frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), and 3) result in publications and academic leadership development. The research will be conducted at the UCLA Department of Neurology with excellent institutional support and opportunities to collaborate. The mechanisms by which mutant tau causes neurodegeneration in FTDP-17 - a group of inherited dementias linked to mutations of the microtubule-associated protein tau - are poorly understood, thereby representing a major knowledge gap in the understanding of cell death pathways in degenerative dementias. The goal of the project is to fill this knowledge gap by focusing on one candidate mechanism, by which tau misexpression may lead to neurodegeneration. We identified this mechanism in previous studies of a Drosophila model of human tau expression. The Aims focus on studies that verify and extend preliminary findings suggesting that GSK-3-beta and beta-catenin, both components of the Wnt signaling pathway, exacerbate mutant tau-induced neurodegeneration related to FTDP-17. Preliminary results suggest that beta-catenin accumulates in CNS regions vulnerable to neurodegeneration and that GSK-3-beta may be sequestered by mutant tau. The applicant will investigate the overall hypothesis that the most common tau mutation, P301L, interferes with the ability of GSK-3-beta to phosphorylate beta-catenin and that the resulting stabilization of beta-catenin triggers enhanced neuronal death. Specifically, correlations of the onset of beta-catenin accumulation and cell death will be addressed. GSK-3-beta activity and association with mutant tau that may lead to beta-catenin accumulation and neurodegeneration will be explored. The proposed biochemical and cell biological studies will initially utilize transgenic mice expressing mutant P301L tau, which model aspects of FTDP-17 clinically and pathologically. Once correlative studies have provided information regarding potential interactions of mutant tau, GSK-3-beta and beta-catenin, functional studies of these interactions are planned. -

Principal Investigator: Wilson, Charles J
Grant Number: 2R37NS037760-06
Title: Neostriatal Cholinergic Interneurons Firing Patterns

Abstract: Altered function of the neostriatal cholinergic interneurons has been implicated in the pathology of Parkinson's disease, Huntington's disease, and a variety of other disorders. The observation that cholinergic antagonists are clinically effective in treating Parkinson's disease has led many investigators to suggest that within the striatum there is a balance of opposing actions of dopamine and acetylcholine. Despite the explosion of information on the pharmacology of acetylcholine in the neostriatum, physiological information has been difficult to obtain due to the rarity of cholinergic interneurons compared to the other cells in the striatum. Using infrared differential interference contrast microscopy, we have recorded from identified cholinergic neurons in slices, and have shown that they are intrinsic pacemakers that exhibit three distinctly different spontaneous filing patterns, even in the absence of fast synaptic input (but with neuromodulators intact). One of the firing patterns resembles that seen in experimental Parkinsonism. This finding provides a window on several otherwise inexplicable observations, including the rhythmic synchronous activity of these neurons in monkeys rendered Parkinsonian by experimental treatment with MPTP. In the proposed experiments, we will employ whole ceil recording of identified cholinergic interneurons and calcium imaging in single cells to determine (1) The ionic mechanisms of the rhythmic bursting firing mode, which most resembles that seen in Parkinsonism, which we already know is related to modulation of calcium and calcium dependent ion channels (2) The basis for synchronization of cholinergic interneurons when they are firing in the bursting mode, including the synaptic connectivity among cholinergic cells and (3) The influence of D1 and D2 dopaminergic agonists and antagonists on the firing patterns of cholinergic interneurons. The effects of dopamine on firing pattern will be directly related to other studies on dopaminergic modulation of specific ion channels to provide an integrated understanding of the actions of dopamine on cholinergic interneurons and the neostriatal circuitry.-

Principal Investigator: WILSON, SCOTT
Grant Number: 1R01NS047533-01
Title: The role of Usp14 in regulating neuronal function

Abstract: The ubiquitin-proteasome system (UPS) is a central pathway common to all eukaryotic cells for regulating protein turnover. There are numerous regulatory pathways that rely on the timely removal of critical proteins. These pathways include the cell cycle, DNA repair, receptor-mediated endocytosis and the induction of long-term memory. The inability to remove unwanted proteins from cells has been linked to several chronic neurological diseases including Parkinson's disease, Alzheimer's disease, and the Spinocerebellar ataxias. While it is clear that these diseases are associated with polyubiquitinated protein aggregates, it is not clear how these aggregates contribute to neuronal dysfunction. In contrast to the polyubiquitination signal that targets proteins for proteasomal degradation, a monoubiquintin tag can signal receptor internalization and sorting of intracellular vesicles. This modification by monoubiquitin is reversible and, akin to phosphorylation, can regulate protein localization and activity. We have recently demonstrated that Uspl4, a deubiquitinating enzyme (DUB) that specifically removes ubiquitin from proteins, is mutated in the neurological mouse mutant ataxia (ax/j). The axJ mice do not show protein aggregation defects or neuronal loss. Instead, these mice exhibit defects in synaptic transmission, indicating that neurological disease may be rooted in synaptic dysfunction. Our working hypothesis is that loss of Uspl4 disrupts the ubiquitinated state of specific components of the neurotransmitter release machinery, thereby resulting in synaptic defects. This proposal is therefore directed at addressing the role of Uspl4 in regulating synaptic function. The first Aim will determine if Usp 14 associates with the 26S proteasome in neurons and if it has a role in ubiquitin-dependent proteolysis. In the second Aim, we will identify components and pathways that are regulated by Usp14 in order to better understand the regulation of ubiquitin modification in normal physiology and disease. The third Specific Aim will determine which neuronal circuits are disrupted by the loss of Uspl4 and examine how these circuits contribute to the tremor, ataxia and muscle wasting phenotypes of the ax J mice. Completion of these Specific Aims will enable us to uncover new processes that rely on ubiquitin-signaling and to determine how alterations in these pathways can lead to neurological disease.-

Principal Investigator: WINDEBANK, ANTHONY J
Grant Number: 5R01NS040471-05
Title: MECHANISMS OF NEURONAL DEATH AND NEUROPROTECTION

Abstract: "Mechanisms of Neuronal Death and Neuroprotection" Chemotherapeutic neurotoxins provide model systems in which basic cellular mechanisms relevant to human disease can be studied. Findings can lead directly to design of treatment trials. Neurotoxicity is dose limiting for cis-diamminedichloroplatinum (cisplatin; CDDP), a first line agent for treating ovarian, testicular, and other neoplasms. The primary target is the dorsal root ganglion (DRG) neuron or its axon. We have demonstrated in a rat model that CDDP induces apoptosis in DRG and have replicated this process in vitro. Nerve growth factor (NGF) prevents this cell death. In cancer cells CDDP binds to DNA. Dividing cells respond to DNA damage by slowing or arresting growth allowing the cell to repair damage before proceeding to DNA replication. If DNA damage is extensive, the cell undergoes apoptosis. The investigators have previously demonstrated that CDDP induces apoptosis in neurons. This is preceded by up regulation of nuclear cyclin D1 expression and increased phosphorylation of the retinoblastoma gene product. These biochemical changes and ceil death are prevented by nerve growth factor (NGF). They propose that DNA damage in neurons induces repair processes that up regulate genes associated with transition from G0 to G1. Since it is highly disadvantageous for post-mitotic neurons to divide, they undergo apoptosis. We will test this hypothesis by (1) determining whether CDDP induces DNA damage by forming Pt-DNA adducts in DRG neurons and whether Pt-DNA complexes are sufficient to induce apoptosis; (2) determining which steps in DNA damage recognition or repair are necessary to initiate cisplatin induced neuronal death using mouse knockouts. If they are necessary, do they occur upstream of the cell cycle changes? (3) Determine where NGF interrupts the death pathway and which NGF signal transduction pathway is responsible for rescue. NGF can be used therapeutically as a specific neuroprotectant. It is one of the primary survival factors for DRG neurons, it has been safely administered to humans, systemic NGF has access to DRG neurons in vivo, and most cancer cells do not have NGF receptors. In the future we will determine whether NGF can be used therapeutically in animal and human models of GDDP neurotoxicity. We will also determine whether the effects of NGF are shared by the other DRG growth factors, brain derived neurotrophic factor (BDNF), and neurotrophin-3 (NT3). -

Principal Investigator: WOLOZIN, BENJAMIN L
Grant Number: 7R01NS041786-05
Title: Ubiquitination/Receptor Signaling--Regulation by Parkin

Abstract: Mutations in the gene coding for Parkin cause a rare familial form of Parkinsonism, autosomal recessive juvenile Parkinsonism, that results in death of dopaminergic neurons in the substantia nigra. To understand how parkin causes disease, we need to understand the regulation and function of parkin. Our studies have lead us to investigate ubiquitination, which is a process that regulates protein degradation. We hypothesize that parkin regulates ubiquitination of other proteins in response to cellular contact with matrix proteins (such as collagen and laminin), and thereby controls regulation of the cytoskeleton and signal transduction by matrix proteins and their integrin receptors. Loss of parkin function could cause neurodegeneration by inhibiting matrix signaling and impairing maintenance of processes by neurons. Our preliminary data support this hypothesis by demonstrating that parkin-dependent ubiquitination is activated by cellular binding to matrix proteins. We have also identified parkin binding proteins that are associated with integrins. Conversely, cell lines that have reduced parkin expression (due to anti-sense parkin cDNA) decrease ubiquitination, retract processes upon cellular exposure to matrix proteins, and have abnormal signal transduction. The goal of this proposal is to investigate the regulation of ubiquitination by parkin (Aim 1), determine the role of parkin in regulating signaling in response to exposure of cells to matrix proteins (Aim 2) and identify common functional deficits associated with disease-related mutations in parkin. Interestingly, parkin is also linked to other forms of neurodegeneration. Parkin binds to alpha-synuclein, and in brains from donors with Parkinson's disease parkin accumulates in inclusions that contain alpha-synuclein, and shows 75% less binding of parkin to two proteins, filamin and hCDCrel2a. We intend to investigate the mechanism of parkin dysfunction by determining how parkin function is altered in Lewy body diseases, and whether oxidation or alpha-synuclein aggregation causes the dysfunction of parkin (Aim 3). The research in this proposal will provide insight into the function of parkin, determine how mutations in parkin produce disease, and provide a new window to understand the molecular pathophysiology of Parkinson's disease.-

Principal Investigator: WOOTEN, GEORGE F
Grant Number: 3P50NS039788-05S1
Title: MITOCHONDRIAL ETIOLOGIES OF PARKINSON'S DISEASE

Abstract: Unavailable

Principal Investigator: XU, ZUOSHANG
Grant Number: 1R01NS048145-01
Title: Understanding mechanism and therapy of ALS using RNAi

Abstract: Diseases caused by dominant, gain-of-function mutations develop in people bearing one mutant and one wild-type copy of the gene. Some of the best known examples of this class are neurodegenerative diseases, including Huntington's, a subset of amyotrophic lateral sclerosis (ALS), Alzheimer's and Parkinson's diseases. In all these diseases, the exact pathways whereby the mutant proteins cause cell degeneration are not entirely clear, but the origin of the cellular toxicity is known to be the mutant protein. Thus, selectively lowering or eliminating the mutant protein is a key step in developing effective therapies. Until recently, it was not clear how specific down-regulation of a wide variety of mutant proteins could be achieved. But now, new advances in RNA interference (RNAi) raise the possibility that RNAi can be developed and eventually applied as a therapeutic means for these neurodegenerative diseases. RNAi can mediate sequence-selective suppression of gene expression in a wide variety of eukaryotes by introducing short RNA duplexes (called small interfering RNAs or siRNAs) with sequence homologies to the target gene. Recent experiments indicate that small hairpin RNAs (shRNAs) transcribed in vivo can trigger degradation of corresponding mRNAs similar to siRNA. These developments raise the possibility that siRNA duplexes or vectors expressing shRNAs may be used to block the expression of a toxic mutant gene. This proposal investigates in vivo efficacy of RNAi therapy using transgenic technology in a mouse model for ALS that is caused by mutations in Cu, Zn superoxide dismutase (SOD1). To determine the potential of RNAi therapy, we will express shRNAs targeting specifically the mutant mRNAs in transgenic mice. We will test how effective and how specific these shRNAs are in suppressing the mutant protein expression and alleviating the disease. To determine in which cell types the suppression of the mutant expression is most crucial, we will express shRNAs in selected cell types using Cre-lox recombination system, We will determine in which cell type suppression of mutant SOD1 expression has the largest impact in alleviating disease. To determine the optimal time for therapy, we will use the Tamoxifeninducible Cre recombinant system to determine at what stage of the disease induction of shRNA to suppress mutant SOD1expresion is most effective.-

Principal Investigator: YOUNG, ANNE B
Grant Number: 3P50NS038372-05S2
Title: MGH/MIT PARKINSONS DISEASE RESEARCH CENTER

Abstract: Unavailable

Principal Investigator: YOUNG, ANNE B
Grant Number: 2P50NS038372-06A1
Title: MGH/MIT MORRIS UDALL CENTER OF EXCELLENCE IN PD RESEARCH

Abstract: The MGH/MIT Morris Udall Center of Excellence in PD Research is taking a broad, collaborative and interactive approach to the study of Parkinson's disease. The Projects address critical questions concerning the selective vulnerability of dopamine neurons, the mechanism and consequences of Lewy body formation and alpha-synuclein aggregation, the neural systems consequences of parkinsonism and synuclein pathology, and molecular approaches for modifying this pathology. These issues will be explored using a range of systems, from yeast genetics, to mammalian cell culture, to rodent models to human postmortem material. The Center incorporates state-of-the-art technologies including high throughput yeast genetic screens to identify modifiers of synuclein aggregation and toxicity, viral vector gene transfer to study factors in mammalian cell culture and rodent models, multi-unit tetrode recordings to study striatal plasticity, fluorescence lifetime imaging to study protein-protein interactions, and laser capture microdissection and gene arrays to study transcriptional dysregulation. The Center has a Clinical and Training Core that provides care to patients with Parkinson's disease, gathers data on clinical features of the disease and response to therapy, solicits brain donations for neuropathological study, and trains outstanding clinician scientists to be future leaders in the field. The Center also has a Bioinformatics Core that serves to integrate and analyze data across the projects, and facilitate sharing of the information. The Administrative Core is charged with management of the Center and facilitating the sharing of information, ideas, and reagents among the investigators and with other components of the Udall Centers consortium. The investigators of the MGH/MIT Center are dedicated to a program of collaborative and interactive studies which will lead to better treatments for people with Parkinson's disease.-

Principal Investigator: ZABETIAN, CYRUS P
Grant Number: 5K08NS044138-03
Title: DBH as a Modifying Gene in Neurodegenerative Diseases

Abstract: The applicant, Dr. Cyrus Zabetian, has spent the past three years as a postdoctoral fellow at Yale University/ VACHS. He will join the neurology faculty at the University of Washington next year where his future mentors, Drs. Thomas Bird and Gerard Schellenberg, have established a superb research program in neurogenetics. His training will include participation in laboratory meetings, seminars, structured courses, and annual scientific meetings. He will become part of a rich collaborative network of researchers with expertise in clinical and molecular neurogenetics, catecholamine biochemistry, and biostatistics. Dr. Zabetian's long-term plans are to become established as an independent laboratory investigator within five years, and remain actively involved in patient care and resident training on the neurology service. In neurodegenerative disease research, identifying genetic mechanisms underlying compensatory changes in surviving neurons promises to lead to improved strategies of diagnosis and treatment. The project proposed in this application seeks to determine if a newly discovered promoter polymorphism (C-1021T) influences regulation of the DBH gene with potential clinical consequences in Parkinson's disease (PD), and is divided into three parts. The goal of part I is to evaluate whether homozygosity for the T allele of C-1021 T, which is associated with low levels of plasma DBH enzyme, is predictive of an earlier onset and more severe symptoms of sympathetic failure in patients with PD. A group of forty subjects homozygous for either the C or T allele will be selected from a population of 400 clinic patients with PD and assessed longitudinally using indices of sympathetic function. Part II seeks to determine whether C-1021T strongly associates with DBH expression in noradrenergic tissues. Levels of DBH protein and mRNA will be compared in postmortem human adrenal medulla specimens, homozygous for either the C or T allele, using western blots and quantitative real time RT-PCR, respectively. Part III will assess whether C-1021T is directly functional. If preliminary results are favorable, two transgenic mouse lines homozygous for either the T or C allele will be created in which the proximal 2 kb of the endogenous mouse DBH promoter is replaced by homologous human sequence. Comparing plasma and tissue levels of DBH protein and catecholamines in the two lines will detect the effect of each allele on DBH expression.-

Principal Investigator: ZASSENHAUS, HANS P
Grant Number: 5R01NS041785-04
Title: Pore opening: A target for mitochondrial DNA mutations

Abstract: Mitochondrial dysfunction is seen not only in late-onset neurodegenerative disease, such as Alzheimer's, Parkinson's, and Huntington's, but with aging in the normal brain as well. Since the frequency of mitochondrial DNA (mtDNA) mutations in the brain climbs hundreds to thousands of fold with age, it is widely thought that such mutations may contribute to cause mitochondrial dysfunction. To experimentally probe their pathophysiology, transgenic mice were constructed that rapidly accumulate specifically mtDNA mutations in cardiomyocytes. These mice reveal that mtDNA mutations - at frequencies commonly seen with age or disease in humans - indeed cause pathology. Characterization of mitochondria from those mice suggests a novel molecular mechanism for the pathogenesis of elevated levels of mtDNA mutations. As mutations rise so do the levels of mutant proteins encoded by the mitochondrial genome. Some of these mutant proteins will misfold. One of the major chaperones catalyzing protein folding in mitochondria is cyclophilin D (CyP-D), a peptidyl-prolyl cis/trans isomerase that also functions to regulate mitochondrial pore transition. Elevated levels of misfolded mitochondrial-encoded proteins are proposed to lead to dysfunction of CyP-D and, in turn, to dysregulation of pore transition. Catastrophic pore transition is known to cause massive disruption of calcium homeostasis in neurons and to signal cell death by apoptosis. To test these hypotheses, we propose to: 1) characterize the structural and functional alteration in CyP-D that occur when the levels of mtDNA mutations rise, 2) determine the basis for the alteration in mitochondrial pore transition that occurs when mutation levels rise, and 3) generate transgenic mice with an accelerated accumulation of mtDNA mutations in the brain to characterize the effect(s) of these mutations on the function of CyP-D and the permeability transition pore in neurons. These studies are broadly significant to understand molecular mechanisms for the pathogenesis of mtDNA mutations. Since such mutations may be an important contributing factor for many adult-onset diseases, these studies may provide insights into novel therapeutic strategies. -

Principal Investigator: ZEITLIN, SCOTT O
Grant Number: 5R01NS043466-02
Title: Loss-of-function mechanisms in Huntington's disease

Abstract: Huntington's disease (HD) is a dominant hereditary neurodegenerative disease that is caused by the expansion of a stretch of CAG repeats within the HD gene that encodes a large protein (huntingtin; htt) of unknown function. HD is thought to be the consequence of a deleterious gain-of-function that is conferred by the expanded stretch of polyglutamine encoded by the CAG repeats. The role of the normal function of htt in the disease process is unknown, but our recent work and that of others suggests that loss of normal htt function may also contribute to pathogenesis. Our long-term objective is to use genetic approaches to understand the role of htt's normal functions in HD pathogenesis using both cell culture and mouse models. To accomplish this objective, we propose three complementary specific aims that are designed to test the potential contribution of different loss-of-function mechanisms in HD. A fourth aim is designed to test a potential therapeutic strategy based on restoring normal htt function in HD mouse models. (1) To test the hypothesis that loss-of-function in HD may occur through mutant htt's ability to sequester wild-type htt via the polyglutamine stretch, we will generate an epitope-tagged allele of the mouse HD gene homologue (Hdh-deltaQ) that lacks precisely the polyglutamine stretch. The ability of this modified version of htt to resist sequestration by mutant htt will be assessed in cell culture. In addition, in order to test if htt is capable of participating in potential dominant-negative interactions by interacting with itself, an ES cell line with targeted insertion of different epitope tags in each Hdh allele will be generated for use in immunoprecipitation pull-down assays. (2) To test if htt loss-of-function may occur through mutant htt's ability to activate caspase-mediated proteolysis, and if proteolytic cleavage of htt is a rate-limiting step in HD pathogenesis, we will compare the onset and progression of phenotypes exhibited by two knockin HD mouse models: the first expressing a full-length mutant htt, and the second expressing a truncated version of mutant htt. Both mutant proteins are expressed under the control of the endogenous Hdh promoter, enabling a direct comparison between the two models. (3) Htt loss-of-function may also occur via dominant-negative interference of wild-type htt interactions with protein partners. To test this hypothesis in vivo, we will characterize the impact of losing htt interactions with the postsynaptic density 95 protein that could lead to altered N-methyl-D-aspartate (NMDA) receptor function in an Hdh conditional knockout mouse model. (4) Finally, we will attempt to rescue phenotypes in an HD mouse model by over-expressing a temporally